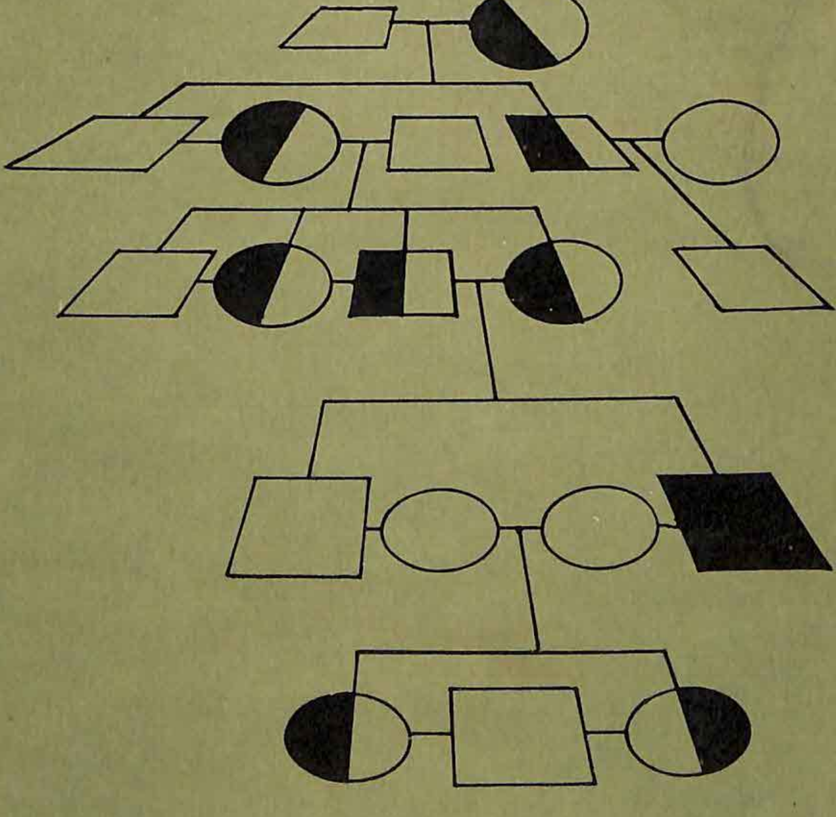
ESSENTIALS OF HUMAN GENETICS



BHATNAGAR · KOTHARI · MEHTA

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PREFACE

Genetics has become an integral part of medical teaching at undergraduate and postgraduate levels. Our interest in the subject coupled with the absence of any suitably compact text encouraged us to write one that covers most of the essentials of human genetics.

For aspiring medical students, who are forever burdened with a heavy load of study, genetics is an interesting but a difficult discipline. It is a science where conceptual and terminological changes occur every day. Any text on medical genetics should be brief, simple, comprehensive and yet interesting enough to sustain a student's keenness for studying it. The authors are happy that in this regard their attempt has met with some success—as demonstrated by the fact that this is the third edition of the book.

The response from students and teachers alike, for the first two editions, has been spontaneous and heartening, and we have received some useful suggestions from them—many of which have been incorporated in this new edition.

This edition is new in more ways than one:

- The entire text has been revised, rewritten and substantially added to.
- The illustrations—designed for ready comprehensibility and reproducibility—have been redrawn and changed at many places.
- A two-column format has been adopted.
- An introductory section has been added to the beginning of each chapter.
- A summary has been added at the end of every chapter.
- Each chapter carries a set of questions that will act as exercises in self-assessment for the student.
- The glossary has been updated.

We have made, in this book, a small attempt to encompass, in brief, the vast and growing field of genetics and the consequent explosion in its literature. This book could provide a starting point in the introduction to a course in human genetics, and we feel that it will play a useful role in today's medical education. We do, however, feel that given the fact that, in advanced countries, genetic disorders are receiving ever-increasing attention, perhaps in India too chairs in medical genetics could be instituted in all medical colleges.

Orient Longman and Vinay Masurekar and Hemang Shah deserve our thanks for as it were co-authoring the book. Orient Longman are responsible for giving the book a new shape and a more attractive layout; Mr. Masurekar has laboured, artistically, over the illustrations. Our renewed thanks are due to Messrs. Kothari Medical Publishing House for their cooperation in the publication of the earlier editions. To colleagues in the teaching fraternity, and to students, we are grateful for providing us with feedback that has assisted the revisions.

SURENDRA BHATNAGAR
MANU KOTHARI
LOPA MEHTA

BOMBAY

Dedicated to
that marvel of all marvels
THE GENE

The greatest single achievement of nature to date was surely the invention of the molecule of DNA. We have had it from the very beginning, built into the first cell to emerge, membranes and all, somewhere in the soupy water of the cooling planet three thousand million years ago. All of today's DNA, strung through all the cells of the earth, is simply an extension and elaboration of that first molecule. In a fundamental sense we cannot claim to have made progress, since the method used for growth and replication is essentially unchanged.

Lewis Thomas
in "The Wonderful Mistake"
from *Notes of a Biology Watcher*

In this book, the current British practice has been followed with respect to the spelling of certain medical terms such as 'Down's syndrome' 'Klinefelter's syndrome' etc. However, the authors wish to clarify that the alternative spellings such as 'Down syndrome' 'Klinefelter syndrome' etc., are equally acceptable.

CONTENTS

1. An Introduction to Human Genetics 1
2. Cytogenetics — Normal Chromosomes 5
3. Cytogenetics — Abnormal Chromosomes 32
4. Molecular Genetics — Genes 50
5. Modes of Inheritance 81
6. Biochemical Genetics 100
7. Immunogenetics including Blood Groups 111
8. Population Genetics 126
9. Genetics and the Doctor — Genetic Counselling 134
10. Experimental Genetics 142

Glossary 151

Bibliography 162

Index 163

1 An Introduction to Human Genetics

The increasing interest in genetics, that teachers, clinicians, researchers and students show, can be attributed to the amazing progress that genetics by itself has shown as a science, and to the applied aspects of genetics which are concerned with the 'why' of a particular disease, rare or common. With minimized infant mortality, and with better nutritional status, more and more persons grow into adulthood and beyond. And with the increasing life-span, there appear such diseases as cancer, diabetes, heart attack and so on. To a parent with, say, cancer or coronary artery disease, there is a dual question: Did he/she inherit the disease? Will she/he pass on the same to the progeny?

While modern genetics, as of today, has hardly any answer to such queries, it is providing some insights that augur well for the future.

* * *

Genetics is the science of heredity. Human genetics deals with the **inherited characters** — physical and mental, normal as well as abnormal — in an individual, a family, a race or a population. It is concerned with the ways in which these characters are transmitted from generation to generation. It also includes the study of hereditary factors, and the ways in which they express themselves during the development and life of an individual.

Beginning of the Science of Genetics

The work of an Austrian monk **Gregor Mendel** in the latter half of the nineteenth century laid the foundation of the science of genetics. Luckily he selected seven contrasting characters in **garden peas** for his work. For each experiment he crossed varieties of pea differing only in one of these characters. He proposed that each **character or trait** was determined by a pair of factors (now known as genes). From his numerous, painstaking studies, he enunciated the principles of heredity, as follows:

1. **The First Law or Law of Segregation of Alleles:** During the formation of gametes, the two members of a single pair of genes (alleles) are never found in the same gamete, but instead, always segregate and pass to different gametes. In an individual, each member of an allelic pair is originally derived from separate parents, one male and the other female. The traits or characters determined by such pairs of alleles do not blend in the offspring, since the alleles retain their identity and can pass unchanged from one generation to the next, irrespective of the expression of a trait in any generation.

2. **The Second Law or Law of Independent Assortment:** This law states that, during gametogenesis, members of different gene-pairs assort randomly or independently of one another.

Mendel's work reported in 1865-1866, remained unnoticed till 1900, when it was independently rediscovered by three biologists: de Vries, Professor of Botany in the University of Amsterdam, Correns, a botanist at the University of Tubingen, and von Tschermak-Seysenegg, an assistant in the agricultural experimental station of Esslingen near Vienna. Thus, development of genetics as a science started not from Mendel's own paper, but from the papers that reported the rediscovery of his laws.

The chromosome theory of inheritance. In 1903 Sutton and Boveri independently proposed the chromosome theory of inheritance. The theory suggested that the chromosomes are the carriers of hereditary factors or genes, and the behaviour of the chromosomes at the time of cell division provides the cytological basis for Mendel's laws of inheritance.

The Growth of Human Genetics

In the 18th and 19th centuries, prior to Mendel's work some simple patterns of inheritance were known and reported. However, there was no understanding of the how and the why of these patterns. The characters which were known to be inherited were very obvious ones like, polydactyly (extra fingers), haemophilia, and colour blindness. Maupertuis of France studied the inheritance of albinism and polydactyly in the 18th century. Otto described haemophilia in a family from New Hampshire in 1803.

Galton, in 1875, made a distinction between the effects of environment and heredity. He suggested the study of twins for finding out the difference between the influence of **heredity** and that of **environment**. Galton also studied the inheritance of physical features and of special talents. He found that qualitative analysis was not a satisfactory method for the study of inheritance of such traits. He initiated quantitative genetics **(polygenic inheritance)** for such traits. He introduced to genetics, the statistical concept of the regression co-efficient. This marked the beginning of the mathematical aspect of human heredity.

Until the beginning of the 20th century, human genetics was essentially concerned with tracing of pedigrees and studies on the effects of marriages between cousins (consanguineous marriages). Then came a series of break-throughs by independent researchers.

In 1902, **Garrod,** who ranks with Galton as a pioneer in human genetics, reported for the first time, alkaptonuria as an example of mendelian inheritance in man. In 1908, Garrod developed his concept of 'the inborn errors of metabolism.' With further understanding of biochemical genetics, this concept was elaborated in 1941 by **Beadle** and **Tatum** who suggested the 'one gene-one enzyme hypothesis.'

Landsteiner in 1900 discovered the ABO blood groups. This discovery initiated the study of the genetics of blood groups.

Hardy, a mathematician at Cambridge University, and **Weinberg,** a physician in Stuttgart, independently propounded a law, the Hardy-Weinberg law, which laid the foundation of **population genetics.**

Galton put forward the idea of hereditary improvement of man and animals by selective breeding, for which he coined the term **eugenics,** an impracticable and hazardous plan for use on human beings as the knowledge of human genetics is as yet elementary.

Most of the diseases known to be genetically transmitted, tended to be fatal in early life. However, with improved medical care, more and more patients with these disorders now survive and manifest the same. The science of genetics has found a useful role in relation to the study of such diseases. This branch of genetics is known as **medical genetics.**

Some of the other significant events in the development of human genetics are:

1. Ford, Haldane and others suggested the role of infectious disease in influencing the genetic constitution of man. Allison in 1954 gave the first clear evidence of such a role, by his study of the relationship of malaria and the gene for sickle haemoglobin.
2. Haldane studied linkage quantitatively in man for the first time by estimating the distance separating the colour blindness and haemophilia loci on the X chromosome. Mohr discovered an autosomal linkage in man by studying the linkage between the Lutheran blood group and the secretor factor.
3. Gerty Cori and Carl Cori in 1952 demonstrated the first specific enzyme defect

in an inborn error of metabolism — deficiency of glucose-6-phosphatase, in one form of the glycogen-storage disease. Jervis, in 1953, detected deficiency of phenylalanine hydroxylase in phenylketonuria (PKU). Garrod's prediction of an enzyme defect in alkaptonuria was confirmed by La Du and his colleagues in 1958.

4. In 1956, Tjio and Levan on the one hand, and Ford and Hamerton on the other, independently showed for the first time that the number of chromosomes in man is 46, and not 48 as was believed till then.

5. Ingram in 1957 detected the role played by a gene in determining the amino acid sequence of a protein. He studied the difference between normal haemoglobin A and sickle haemoglobin of man.

6. Lejeune and his colleagues, in 1959 discovered for the first time a chromosomal aberration, providing the basis for a congenital malformation in man—mongoloid idiocy (also called Down's syndrome).

7. Ford and Jacobs and their colleagues in 1959 established the role of the Y chromosome in sex determination in man.

8. Nowell and Hungerford, in 1959 for the first time identified the specific chromosomal aberration associated with cancer—in this case, chronic myeloid leukaemia (CML).

9. Several researchers simultaneously suggested in 1961 that one X chromosome in the normal female is relatively inactive genetically (the Lyon hypothesis).

Genetics is one of the most recent and rapidly advancing sciences and many a Nobel prize in Physiology and Medicine has been awarded to geneticists.

1930	Karl Landsteiner for research on blood groups and immunology.
1933	Thomas Hunt Morgan for research on the nature of the gene.
1946	Herman Joseph Muller for the discovery of the induction of mutation by X-rays.
1958	George Wells Beadle and Edward Lawrie Tatum for contributions in biochemical genetics; and Joshua Lederberg, for the discovery of sexual recombination in bacteria.
1959	Arthur Kornberg and Severo Ochoa, for studies of the chemistry of DNA and RNA.
1961	James Watson, Francis Crick and Maurice Wilkins for elucidation of the structure of DNA.
1965	Francois Jacob, Andre Lwoff, and Jacques Monod, for their discoveries concerning genetic control of enzyme and virus synthesis.
1968	Marshall Nirenberg, Har Gobind Khorana, and Robert Holley, for "cracking the genetic code" and determining the means by which a gene determines the sequence of amino acids in a protein.
1975	Howard Temin and David Baltimore, for challenging the central dogma which hitherto denied the possibility of cytoplasmic RNA directing nuclear DNA synthesis.
1977	Hamilton Smith, Daniel Nathans and Werner Arber for work with restriction enzymes used in DNA research.
1983	Barbara McClintock for her discovery that genes can move from one spot to another on chromosomes of plant and change future generations of plant they produce, thereby introducing the concept of "jumping genes".
1984	Niels Jerne, Ceśar Milstein and Georges Köhler for the concept and the production of "monoclonal antibodies".

It is convenient to divide the subject of human genetics into the following interrelated parts:

1. **Cytogenetics** — A study of chromosomes — normal and abnormal.
2. **Molecular genetics** — a study of the molecular structure of the genetic material and its functional significance in the normal and abnormal state. It also includes the genetic control of

embryonic development — **developmental genetics.**

3. Modes of inheritance of genes amongst family groups.
4. **Biochemical genetics,** including the genetics of haemoglobin, inborn errors of metabolism, and **immunogenetics.**
5. **Population genetics.**
6. **Genetic counselling,** the application of genetic principles in diagnosis, prevention and treatment of human genetic disorders. This can also be called "applied medical genetics".
7. **Experimental genetics.**

SUMMARY

Genetics: The study of inherited traits and their transmission from generation to generation.

Mendel (1866): Through his garden-pea experiments laid the foundation for a scientific study of this discipline. His laws of 'segregation' and 'random or independent assortment' are a by-word in genetics.

Sutton and Boveri (1903): Suggested chromosomes as the carriers of hereditary factors or genes.

Galton (1875): Studied identical and fraternal twins to assess the relative influences of heredity and environment.

Garrod (1902): Initiated the concept of 'inborn errors of metabolism'.

Landsteiner (1900): His discovery of ABO blood group system pioneered blood transfusion and study of genetics of blood groups.

Other noteworthy landmarks in progress of human genetics are:

1. demonstration of chromosomes under the microscope and the study of chromosomal aberrations;
2. Lyon's hypothesis of 'X' chromosome inactivation;
3. elucidation of the structure of DNA by Watson and Crick;
4. cracking of the genetic code by Nirenberg, Khorana and others, and since then;
5. detailed direct observations on DNA with the use of 'restriction endonuclease' enzyme systems.

QUESTIONS

1. State and explain Mendel's laws of heredity.
2. What is meant by the chromosome theory of inheritance?
3. Write a note on the development of human genetics.

2 Cytogenetics – Normal Chromosomes

INTRODUCTION

Chromosomes are the vehicles of heredity. They are passed on to subsequent generations by the process of fertilization, i.e., the union of mature gametes, the sperm and the ovum.

Chromosomes reside in the nucleus of a cell in eukaryotes, except during cell divisions, **mitosis** and **meiosis**, (reduction division) at which time, the nuclear membrane disappears. Their number in each cell is species-specific and they are definite structural entities in their own right.

The human sperm and the ovum contain half the species-specific number of chromosomes. This is called the **haploid** or 1N number and is 23. After fertilization, the zygote comes to possess the **diploid** or 2N number. Except the sex-determining chromosomes designated X and Y, the others exist as matching or homologous pairs and are called **autosomes.**

Each chromosome, essentially, is a DNA-protein package, capable of self-replication; and retains its structural and functional integrity through all successive divisions of any cell type.

The so-called units of heredity or genes are said to occupy specific or fixed positions along the length of a chromosome. These positions are called **gene-loci.**

The chromosomal constitution of an organism is called its **karyotype.** The human karyotype for the male is, 44+XY and that for the female is, 44+XX. Chromosomes are classified on the basis of their length and the position of their **centromere, primary constriction** or **kinetochore**. This is the site which provides attachment at the cellular microtubular apparatus during mitosis and meiosis.

It it now relatively simple to obtain chromosomes for direct study from a wide variety of cells. These 'karyotyping' procedures include an array of staining techniques which bring out the intrinsic cross-banded organization of each chromosome. The stains employed are either the derivatives of quinacrine, a fluorescent dye or the Giemsa dye. Gross chromosomal abnormalities of number and structure can be detected thereby.

All chromosomes play an important role in maintaining an individual and the species. The Y chromosome determines maleness. The presence of at least one X chromosome is essential for survival, as it carries genes concerned with a variety of vital functions. Since, the female has two X chromosomes, she ought to be 'doubly' viable, but that is not so. It was postulated by Mary Lyon, that one of the two X chromosomes in the mammalian female is somehow rendered inactive in each cell of the body. The presence of an inactive clump of chromatin in female nuclei was shown by Barr and Bertram in 1949. The Lyon hypothesis seeks to explain this difference between the male and female nuclei.

* * *

Ovum and Sperm

Parents transmit the hereditary material to the offspring through the two cells, the ovum and the sperm. The human ovum, about 140 μ in diameter and weighing 0 0015 mg. has a large vesicular nucleus (the germinal centre) with a prominent nucleolus (the germinal spot). Its cytoplasm contains yolk granules and is enveloped by a cell membrane known as the vitelline membrane. The ovum is surrounded by a thick, refractile capsule called the zona pellucida. The mature human sperm is an actively motile, freely swimming, elongated cell measuring from 60 μ to 75 μ in length. It consists of a head largely occupied by the nucleus, and a tail which is highly specialized for motility and for orientation of the sperm, preparatory to penetration of the various coverings of the ovum, before fertilization could occur.

Fertilization

Fertilization is the union of a mature ovum and a single, mature sperm to form the zygote, from which an entire individual is produced. Though the ovum and the sperm differ greatly in size, the hereditary influences of the mother and father are nearly equal. The cytoplasm of the ovum is not held responsible for hereditary transmission, but the nuclei which are similar in the ovum and the sperm are regarded as the material carriers of the hereditary contributions of the parents. The nucleus of a zygote, like that of any other cell, appears like an undifferentiated vesicle. It shows the presence of well-defined structures known as chromosomes when the cell divides. Between cell divisions, the chromosomes are usually highly extended filaments that form a diffuse network collectively referred to as **chromatin**. The chromatin may be seen as dispersed and poorly staining **euchromatin** or as clumped and densely staining **heterochromatin.** In most somatic cells there is a mixture of the two states of chromatin, the heterochromatin lying close against the inside of the nuclear membrane and the euchromatin in the central region of the nucleus. Heterochromatin is distinguished into constitutive heterochromatin and facultative heterochromatin. Constitutive heterochromatin is a normal feature of several chromosomes. Inactive X chromosome in female somatic cells represents facultative heterochromatin.

THE HUMAN CHROMOSOMES

Chromosomes are rod-shaped, V-shaped, or J-shaped organelles, present in the nucleus. They are distinctly visible only during cell division. Each chromosome is endowed with individuality, special organization and function. Each is capable of self-reproduction and of maintaining its morphologic and physiologic properties through successive cell divisions. Chromosomes are made up of deoxyribonucleic acid (DNA) and proteins. DNA segments are the genes or the units of heredity.

Each species has a characteristic chromosome configuration, i.e., karyotype, with respect to chromosome number, length, shape, and the nature and sequence of the genes carried by each chromosome.

Till 1956, the human chromosome number was believed to be 48, when it was demonstrated beyond doubt that the human chromosome number is 46. These 46 chromosomes constitute 23 homologous pairs. Each parent contributes to an offspring 23 chromosomes, one member of each pair. Homologous chromosomes look alike and have at identical positions the same gene-loci, determining the same characteristic. However, the genes forming a pair, though determining the same characteristic or trait, are not necessarily identical.

The term haploid refers to a single set of all the chromosomes found in normal gametes, sperm or ovum (Symbol: 1N). In man, N = 23. The zygote, formed by the fertilization of an ovum by a sperm, has therefore 23 pairs of chromosomes. This constitutes, in the zygote and all the normal somatic cells derived therefrom, the diploid state (Symbol : 2 N). A chromosome number which differs by one or more from an exact multiple of the haploid number, e.g., $2\,N - 1$ or $2\,N + 1$, where N is the haploid number of chromosomes, is referred to as aneuploid. **Polyploid** means any multiple of the basic haploid chromosome number, other than the diploid; thus 3 N, 4 N and so on.

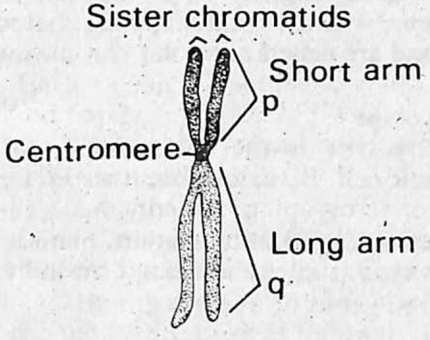


Fig. 1

Components of a chromosome as seen in metaphase. Note that a chromosome is made up of two sister chromatids, which separate during anaphase by splitting of the centromere. After that each sister chromatid is known as a chromosome, made up of one short arm, one long arm and a centromere. As a chromosome is best visible during metaphase, it is always drawn showing two sister chromatids stuck together at the centromere. In the remaining phases of the cell cycle, a chromosome extends outwards and hence it cannot be seen distinctly as a chromosome.

There are two types of chromosomes, autosomes and sex chromosomes. **Sex chromosomes** are a pair of chromosomes which are responsible for sex determination. They differ in males and females. The two sex chromosomes in a female are similar and are designated as XX. The two sex chromosomes in a male are different from one another and are designated as XY. The X of the male is similar to the X of the female, while the Y is much smaller in size than the X. The remaining 22 pairs are alike in the male and in the female and hence are called autosomes. Since females are XX, all ova carry an X chromosome whereas males are XY and hence produce X-bearing and Y-bearing sperms in approximately equal numbers. Because of this, the human female is referred to as the **homogametic sex** (producing only the single type of X-bearing ova); the human male is referred to as the **heterogametic sex** (producing equal number of X-bearing and Y-bearing sperms). In birds, and many insects this order is reversed – the female is heterogametic and the male is homogametic.

Chromosome Structure

Each pair of homologous chromosomes shows a common basic structure during cell division. Each chromosome consists of two parallel and identical filaments, **chromatids,** held together at a narrowed region, the **primary constriction,** within which is a pale-staining region, the centromere or kinetochore

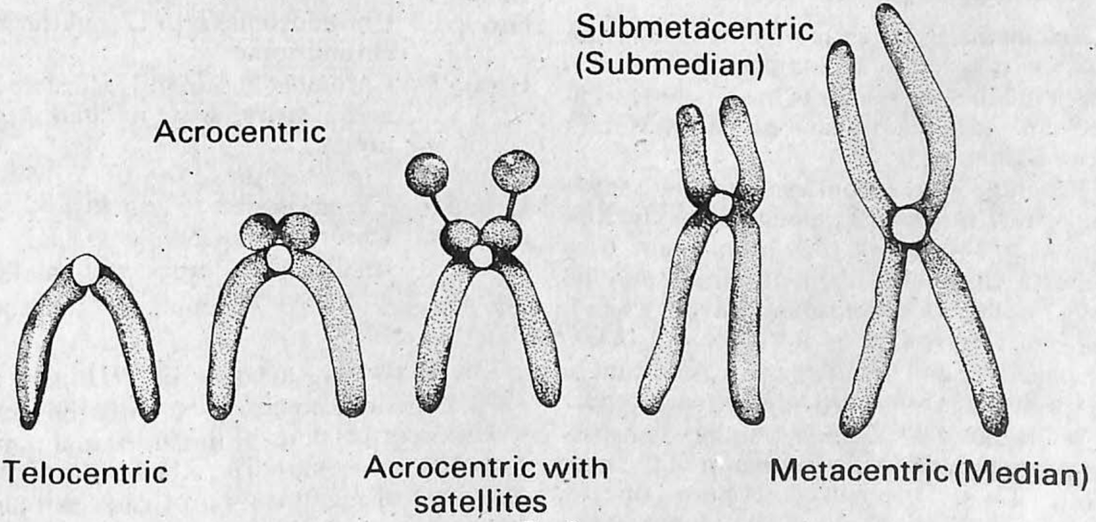


Fig. 2

Types of chromosomes as seen in metaphase. Telocentric chromosomes are not found in man.

(Fig. 1). A chromosome becomes attached to the spindle during cell division by means of the kinetochore. Each chromatid shows two arms, one on either side of the centromere.

Chromosomes are classified into four types depending on the position of the centromere (Fig.2). (1) **Telocentric** chromosomes, wherein the centromere is situated at the end, have only one arm. (2) **Acrocentric** chromosomes, wherein the centromere is situated near the end, have one very short and the other a very long arm. (3) **Submetacentric** chromosomes wherein the centromere is situated somewhere between the midpoint and the end of the chromosome, making one arm of the chromosome short and the other arm long, (4) **Metacentric** chromosomes wherein the centromere is situated near the centre of the chromosome, making both arms almost equal.

Secondary constrictions, found along the length of a chromosome, may be short or long, and are distinguishable from the primary constriction, by the absence of any marked angular deviation of the chromosomal segments. Secondary constrictions are constant in their position and extent. They provide a useful criterion in identifying a particular chromosome in a set.

Telomere. Each extremity of a chromosome is referred to as telomere. It has been observed that the telomere has a polarity that prevents other segments of chromosomes from joining with it.

Satellite. Some chromosomes show a satellite which is a round, elongated body connected to the rest of the chromosome by a delicate chromatin filament, which may be long or short. Chromosomes having a satellite are referred to as SAT-chromosomes. The satellite and the filament are constant in shape and size for a particular chromosome.

Nucleolar zone. Some secondary constrictions are related to the formation of the nucleoli. These specialized regions of the chromosomes are the nucleolar zones or the nucleolar organizers. There are five chromosomes (Number 13, 14, 15, 21, 22) in each nucleus, which have this special characteristic, and are called nucleolar chromosomes.

Karyotype

Karyotype is the chromosome set of a somatic cell. By extension, it also refers to a photomicrograph of an individual's chromosomes arranged in a standard manner. The karyotype is characteristic of an individual, species, genus or a larger group.

Classification of Chromosomes

Chromosomes are identified by their length, the position of the centromere, the presence of satellite bodies, and other morphological characteristics. Now it is a standard practice to number the autosomal pairs in decreasing order of size from 1 to 22 for their classification (the Denver-London system). The sex chromosomes retain their distinctive labels (X and Y). An alternative system is the Denver classification where all the chromosomes, including the sex chromosomes, are assigned the groups from A to G, in order of decreasing length.

Group A	Chromosomes 1, 2, and 3
Group B	Chromosomes 4 and 5
Group C	Chromosomes 6 to 12 and the X chromosome
Group D	Chromosomes 13 to 15 (the larger acrocentrics, with satellited short arms)
Group E	Chromosomes 16 to 18
Group F	Chromosomes 19 and 20
Group G	Chromosomes 21 and 22 (the smaller acrocentrics, with satellited short arms) and the Y chromosome

The **Paris nomenclature** of 1971, and of 1975, has added greater accuracy to the identification of portions of the individual numbered chromosomes. The scheme allows the unequivocal identification of each chromosome by its **banding pattern,** and gives number to the bands of each chromosome.

The short (p) and the long (q) arms are thereby considered to be made up of regions numbered 1, 2, etc. starting at the centromere. Each region is then subdivided into numbered bands, e.g. 2-q, 2, 1 would mean band number 1 in the second region of the long arm of chromosome 2.

Symbols for Chromosomal Nomenclature

The internationally accepted symbols to denote certain normal and abnormal chromosomal features are as follows:

46 XX	normal female karyotype
46 XY	normal male karyotype
A-G	The chromosome groups
1-22	The autosome numbers
X, Y	The sex chromosomes

Diagonal lines indicate **mosaicism,** e.g., 46/47 designates a mosaic with 46-chromosomes and 47-chromosomes cell lines.

Key to Abbreviations

del	Deletion
der	Derivative of chromosome
dup	Duplication
i	Isochromosome
ins	Insertion
inv	Inversion
p	Short arm of chromosome
q	Long arm of chromosome
r	Ring chromosome
s	Satellite
t	Translocation rep: Reciprocal translocation rob: Robertsonian translocation tan: Tandem translocation
ter	Terminal (may be specified as pter or qter)
→	From → to
+ or –	Placed before the chromosome number, these symbols indicate addition (+) or loss (−) of a whole chromosome: e.g., +21 indicates an extra chromosome 21, as in Down syndrome. Placed after the chromosome number, these symbols indicate increase or decrease in the length of a chromosome part; e.g., 5p– indicates loss of part of the short arm of chromosome 5, as in *cri du chat* syndrome.

Preparation of Chromosomes for Study

For chromosome analysis, those cells are chosen which grow and divide rapidly in culture. The most commonly used tissues are skin fibroblasts, bone marrow and peripheral blood cells, and amniotic fluid cells from pregnant women. Viable cells can also be obtained within a few hours after the death of an individual or following spontaneous abortion of an embryo.

From peripheral blood, the leukocytes are separated and added to a small volume of nutrient medium containing **phytohemagglutinin,** which stimulates the leukocytes to divide. The cells are cultured under sterile conditions at 37°C for about three days. During this period the cells divide and then a small amount of **colchicine** is added to each culture. Colchicine prevents the formation of the spindle and stops mitosis at the stage of **metaphase** (explained later). After an hour

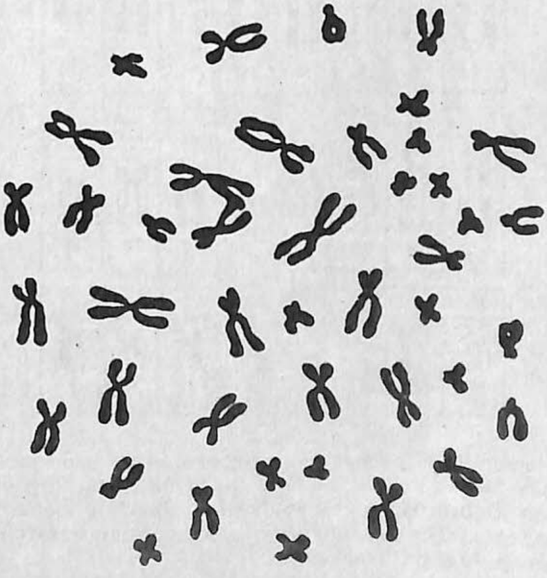


Fig. 3

Human chromosome spread – karyotype – highly magnified.

or so a hypotonic saline solution is added. This makes the cells swell and the chromosomes spread out, which otherwise would remain clumped together. This is followed by taking a high power photomicrograph. (Fig.3). Later the individual chromosomes are cut out from the photograph, arranged in pairs and in decreasing order of size and numbered 1 to 22 with the two sex chromosomes indicated separately. (Fig.4). The resulting arrangement is the karyotype. The process is called **karyotyping,** and it reveals the number and gross structure of chromosomes. However, it is difficult to detect small structural abnormalities. The newer techniques for chromosome study mentioned below have improved the identification of individual chromosomes (Figs.4, 5), and of specific chromosomal aberrations.

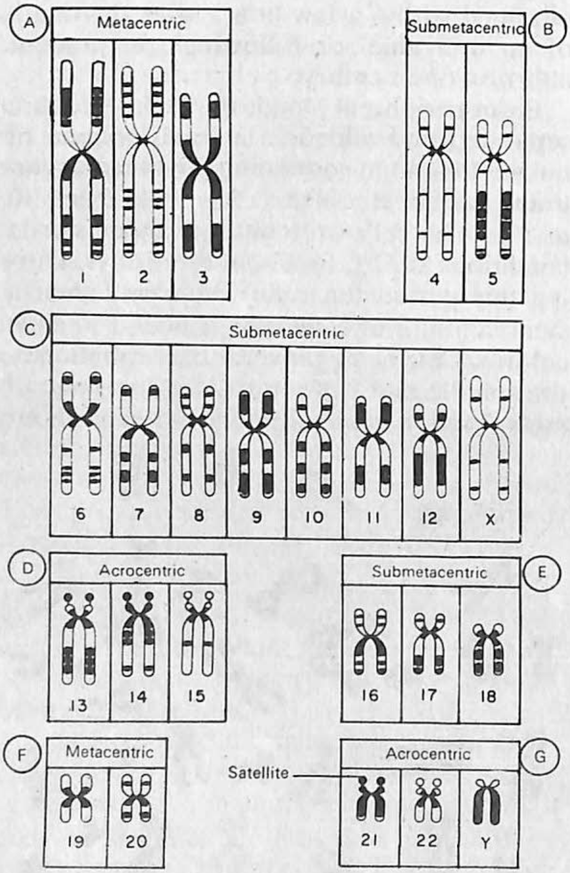


Fig. 4
Human chromosomes, one member of each homologous pair, shown in groups A to G, and in numerical order of the Denver-London classification. Banding pattern indicated. The sex chromosomes X and Y are shown in groups C and G respectively.

Methods for Study of Chromosomes

Chromosome Banding

A number of techniques demonstrate the banded appearance of chromosomes. While the general banding techniques show the full range of bands throughout the chromosomal complement, special banding techniques produce a restricted staining of one or the other subset of bands.

Chromosome banding was discovered by T. Caspersson when he stained chromosomes with the DNA-binding **fluorescent** compound **quinacrine mustard.** Different regions along the length of each chromosome show a varied intensity of fluorescence, so that the chromosomes can be individually identified. Identical Q-banding patterns are present in the meiotic chromosomes. They are especially clear in the long chromosomes at the pachytene stage of prophase. The DNA in the quinacrine-positive Q-bands has distinctive characteristics.

Q banding (Quinacrine Banding). This involves the use of fluorescent stains such as quinacrine hydrochloride or quinacrine mustard. When examined by flurorescence microscopy, each pair stains in a specific manner of bright and dim bands (Q bands). Meiotic and mitotic chromosomes present identical banding patterns.

The Giemsa stain also produces almost identical banding patterns, when used on variously pre-treated chromosome preparations. The **Giemsa (G)-banding** pattern differs from Q-banding in one respect: G-banding shows up a single additional band near the centromeres of chromosome 1 and 16 that is not seen by Q-banding. The G-banding pattern is the same in mitosis and meiosis.

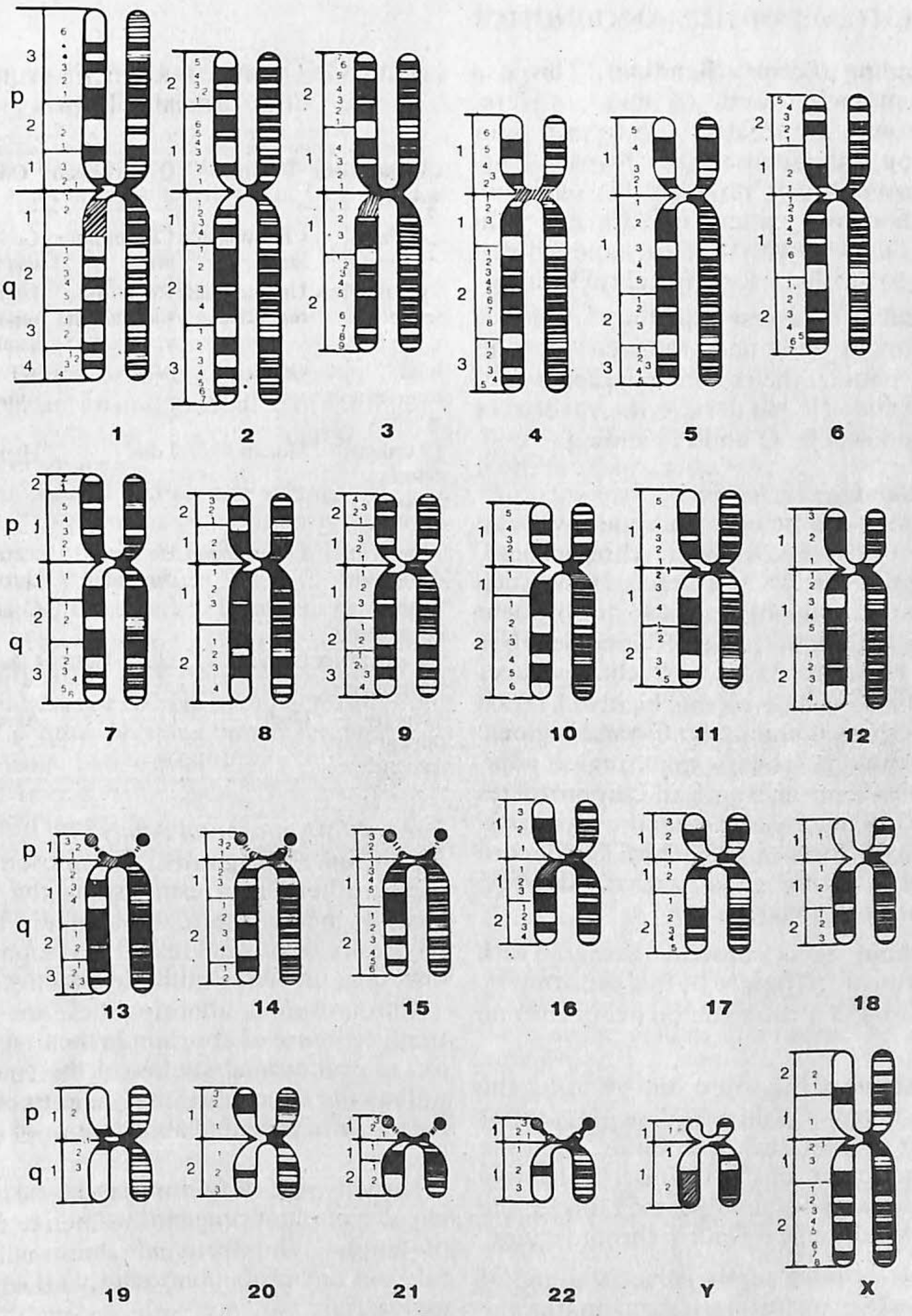


Fig. 5

Human chromosomes showing the banding patterns and numbering, as adopted at the Paris conference 1971. Note that black areas represent positive Q and G bands and negative R bands. The white areas represent negative or pale-staining Q and G bands and positive R bands. The shaded areas represent variable bands.

Note that the short and the long arms 'p' and 'q' are made up of regions numbered 1, 2, etc., starting at the centromere Each region is then subdivided into numbered bands, e.g., 13-p, 1, 2 would mean band number 2 in the first region of the short arm of chromosome 13.

G Banding (Giemsa Banding). This is a very commonly used technique. Here, chromosomes are treated with trypsin prior to staining with Giemsa stain. Trypsin denatures chromosomal protein. The chromosomes stain in a pattern of dark and light bands (G bands), with the dark bands corresponding to the fluorescent (bright) Q bands.

R Banding (Reverse Banding). When a pre-treatment with heat is given prior to Giemsa staining, the resulting dark and light stained bands (R bands) are the reverse of those produced by Q and G banding.

Special Banding Techniques

C-banding can be brought about by alkali extraction of DNA from the chromosome. Highly repetitive DNA sequences resist such extraction. C-banding can also be brought about by staining with the AT-specific DNA ligand, Hoechst 33258, which produces intense fluorescence of the highly AT-rich satellite DNAs found in the C-band regions. These techniques stain a small region adjacent to the centromere of all chromosomes and in addition they also stain the distal portion of the Y chromosome. The C bands contain highly repetitive, non-transcribed DNA, called heterochromatin.

C_d-banding stains a protein associated with the centromere. The site of this centromeric dot or C_d-band is the same on every chromo some.

G 11-staining is carried out by using the Giemsa stain at a highly alkaline pH, i.e., 11 or higher. Under such conditions, only the eosin component of the Giemsa is effective and produces red staining of proteins on the short arms of the acrocentric chromosomes.

AgNOR staining or the silver staining of the nucleolus organizing regions marks the sites of transcriptionally active ribosomal RNA genes. Usually present on six to eight of the D and G group chromosomes, it may be on as few as three, or as high as on all ten of them.

TABLE 1: CHARACTERISTIC FEATURES OF CHROMOSOMAL BANDS

Characteristic Feature	R-bands	Q- or G-bands	C-bands
Location	Chromosome arms	Chromosome arms	Centromeres, distal Y
Type of DNA sequences	Unique, some repetitive	Repetitive, some unique	Highly repetitive satellite
Base composition of DNA	GC-rich	AT-rich	AT-rich, some GC-rich
5-Methylcytosine content of DNA	Moderate	Low	High
Type of chromatin	Euchromatin	Heterochromatin	Heterochromatin
State in interphase	Extended	Condensed	Condensed
Time of DNA replication	Early S	Mid to late S	Late S
Transcriptional activity	High	Low	Absent

Uses of Chromosomal Analysis

1. **Clinical diagnosis.** Chromosomal studies are helpful in establishing the genetic basis of mental retardation, failure to grow physically, abnormal sexual development, or disorders involving multiple systems.

Chromosomal abnormalities are a very frequent cause of abortion in the first trimester. Chromosomal studies of the couple, as well as the conceptus, may help to establish the cause of repeated abortions or of infertility.

Karyotyping of foetus may be carried out in cases of older pregnant women or in cases of familial chromosomal abnormalities to rule out any gross abnormality affecting the foetus.

Studies concerning the association of chromosomal abnormalities with cancer are afoot. It has been shown that in cases of **chronic myelogenous leukaemia** there is an occurrence of the **Philadelphia chromosome**

(Ph^1). It is an abnormal chromosome 22. The distal segment of chromosome 22 (22q−) is translocated to the long arm of chromosome 9 (9q+). **Retinoblastoma** seems to be commonly associated with deletion of a specific segment of chromosome 13, and a specific abnormality of chromosome 14 is seen in various kinds of lymphoma.

2. **Study of genetics.** Chromosome studies enable the assignment of specific positions to human genes on the chromosomes. They also facilitate an understanding of the **linkage** between a disease, specific (marker) gene and other genes.

Chromosomal Polymorphism. Minor heritable differences in banding pattern are seen frequently, especially for chromosomes 1,9,16 and Y. These morphologic variations (polymorphisms) may be useful to trace individual chromosomes through families. Polymorphisms may be used as markers in family studies or to help in the determination of the source of the abnormal gamete in chromosomal abnormalities.

Sex Chromosomes

The X chromosome, a member of group C, is a submetacentric chromosome. Genes present on X chromosome are said to be X-linked or sex-linked. About 100 X-linked traits have been identified so far, in human beings.

The Y chromosome, a member of group G, is a small acrocentric chromosome. It is distinguished from the other members of that group (21 and 22) by its slightly greater length, less widely flaring arms and absence of satellites on its short arm. The Y chromosome is one of the chromosomes which give vivid fluorescence with quinacrine stain both in interphase and in metaphase.

The Y chromosome contains a large block of heterochromatin whose DNA is not transcribed. Its functional genes strongly determine maleness. The male-determining factor is presumably mediated by one or more genes, such as the one required for expression of the **H-Y antigen,** which plays a decisive role in gonadal differentiation. Mutation, deletion or translocation of this gene can affect sex differentiation, leading to such conditions as XY females and XX males.

Polymorphism of the Y chromosome is common; about 10 per cent of males have a Y that is longer or shorter than usual, the difference being entirely in the length of the fluorescent portion. This trait is hereditary.

The sex chromosomes can be thought of as composed of a homologous and a non-homologous or a differential region. The homologous region corresponds to the pairing segment, and when recombination takes place, it is limited to this region. The non-homologous region influences sex determination. Since in meiotic metaphase figures X and Y chromosomes show end-to-end pairing, it is likely that they may have homologous regions at the pairing ends.

In human beings, the Y chromosome determines the male sex. Normally anyone who has a Y is a phenotypic male. An XO individual (lacking the Y chromosome) resembles a female but lacks functional ovaries (Turner's syndrome). It would be appreciated that since the male is heterogametic transmitting either X or Y, the sex of the child depends upon whether the father contributes an X or a Y. The female being homogametic and contributing only an X, cannot determine the sex of the offspring.

Sex Chromatin

Barr and Bertram in 1949 discovered a small chromatin body in the nuclei of neurones of female cats. Similar observations were then extended to neuronal and other tissues of man and animals. The chromatin body is also called the **sex chromatin** or **Barr body.** The frequency with which the sex chromatin can be detected in the females, varies from tissue to tissue. In nervous tissue the frequency may be 85 per cent, whereas in whole mounts of amniotic or chorionic epithelium, it may be as high as 96 per cent. In oral smears the frequency varies between 20 and 50 per cent in normal females.

Sex chromatin is seen as a small chromocentre heavily stained with basic dyes in the interphase nucleus. As shown in Fig. 6, it can be found attached to the nucleolus, as in nerve cells of certain species; attached to the nuclear membrane, as in cells of the epidermis or of the oral mucosa; and seen as a nuclear expansion, in about 3 per cent of neutrophil leucocytes, forming a small rod called the drumstick. It may be also seen free in the nucleoplasm, as in neurones after electric stimulation.

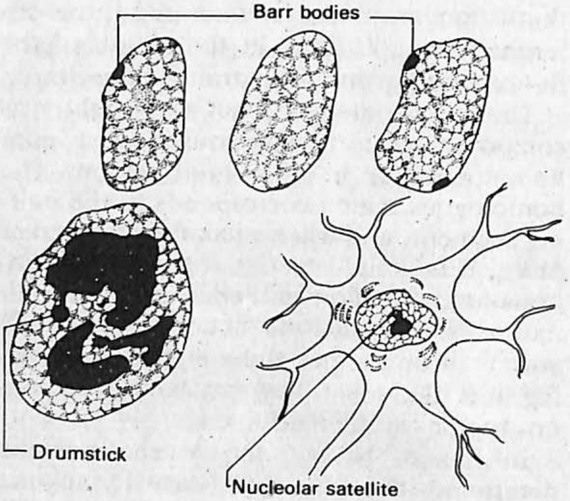


Fig. 6

Sex chromatin (Baar body) as seen in nuclei of epithelial cells, leukocytes and neurones.

The tests for nuclear sex determination include the detection of drumstick in leucocytes and the Barr body in the cells of the epidermis, the oral mucosa and amniotic fluid. The study of the sex chromatin is widely used in medicine. It helps to link certain congenital diseases with sex chromosomal anomalies; among these is the ascertaining of the sex in an intersex during foetal and postnatal life.

Sex chromatin is derived from one of the two X chromosomes which is dark-staining (heteropyknotic) at interphase. The other X is euchromatic. The number of sex chromatin bodies at interphase is equal to nX–1. This means that there is one Barr body fewer than the number of X chromosomes. This relationship between sex chromatin and sex chromosomes is particularly evident in those humans who have an abnormal number of sex chromosomes. The Barr body exhibits alteration in size, if there is any structural aberration of the X chromosome, such as deletion, ring or an isochromosome X (see later). The abnormal X chromosome always forms the sex chromatin and the presence of an unusually small or large Barr body in the buccal smear, indicates the presence of a structural variant of the X.

Barr bodies are absent from oocytes and female germ cells, and only appear at about the 12th day of gestation in extra-embryonic membranes, and by about the 16th day in cells of the embryo, with some variation from tissue to tissue.

It has been observed that at the time of cell division one of the X chromosomes completes its replication of DNA later than the other, and is usually located peripherally, in the region of the nucleus where the sex chromatin is found. The late replicating X, which is condensed and out of phase with the rest of the chromosome set, forms the sex chromatin.

The Lyon Hypothesis

This hypothesis is named after Mary F. Lyon, who stated it first — explicitly and in detail. The hypothesis states that in the somatic cells of female mammals, only one X chromosome is active. The second X chromosome is condensed and inactive, and appears in interphase cells as the sex chromatin. Inactivation of the chromosome occurs in embryonic life and is random, i.e., the inactive X can either be the paternal or the maternal one (X_P or X_M) in different cells of the same individual. However, after the decision has been made regarding which X would be inactivated in a particular cell, all the clonal descendants of that cell will show the same inactive X. Thus, inactivation is random; but once it has occurred, it remains fixed.

The Lyon hypothesis rests on the following observations.

1. The females have the Barr body and the males do not have it.

2. In mice, genes determining fur colouration are of two types: autosomal and sex-linked. Autosomal genes always give uniform colouration to the mouse fur (say dark brown for dominant, white for recessive, the heterozygotes manifesting an uniform light brown in-between dark brown and white).

Sex-linked genes are **X-linked genes.** In a male mouse, there would always be only one X chromosome and thereby the X-linked gene manifests itself phenotypically as a uniform colouration of the fur, either brown or white, depending on the genotype. If the genes are homozygous, then in a female mouse having two X chromosomes, the result is also a uniform dark brown fur colouration or uniform white colouration. If the mouse has only one X chromosome with missing X or Y chromosomes, then also the fur presents uniform dark brown or white colouration depending on the genotype.

However, some surprising patterns spring up in females who have the heterozygous genotype. Here one would expect the uniform dark brown or white colouration of the homozygous phenotype or the intermediate between homozygous (dominant and recessive) i.e., a uniform light brown colour; but what is found is that the coat is not uniformly coloured. The coat shows dark brown and white patches randomly distributed all over the body. This could be only accounted for by the phenomenon of **Random Lyonization** that renders an X chromosome (derived either from the mother or the father) inactive in all cells of the body differently.

3. The number of sex chromatin bodies in interphase cells is always one less than the number of X chromosomes seen at metaphase.

4. During cell division, one X completes its replication later than the rest of the chromosome complement and the late-replicating X is located in the area where the sex chromatin is found.

These observations indicated that the sex chromatin is a condensed, dark-staining (heteropyknotic) X chromosome, and that only one X is active in cellular metabolism; the other X, chosen randomly, appearing as the sex chromatin body. In the male, the single X is uncoiled and active at all times, and consequently there is no sex chromatin.

Evidence for the Lyon hypothesis

1. **Dosage compensation.** The amounts of enzyme such as G6PD or antihaemophilic globulin, which are produced by genes located on the X chromosome are equal in the two sexes. Inactivation of one of the two chromosomes explains this.

2. **Variability of expression in heterozygous females.** Females, with an abnormal X-linked gene present on only one X-chromosome, i.e., heterozygous, do express abnormalities when there is a **Total Lyonization,** whereby the abnormal gene is functional in a majority of the cells.

3. **Mosaicism.** Women show mosaicism at cellular level, having two populations of cells, one population with one X active, the other with the alternative X active. In tissue cultures, two different clonal populations can be demonstrated.

There are some facts that do not conform to the Lyon hypothesis. The most striking of these is the abnormal phenotype of XO and XXY individuals, who physically differ from normal XX females or XY males, respectively. If one X chromosome is inactivated, XO females should be normal since XX normal females are effectively XO, and XXY males should be normal as they are effectively XY as in normal males. It is possible to reconcile these facts with the hypothesis, if it is assumed that sexual differentiation is influenced at a very early embryonic stage prior to the inactivation of an X chromosome to form the Barr body. In that case, XO and XX, and XXY and XY are not equivalent in terms of developmental control. Another possibility is that if inactivation does not affect a whole X-chromosome but only part of it, and if the non-inactivated section carries genes that determine the phenotypic difference between XO and XX, and between XXY and XY, then XX is not equivalent to XO, and XXY is not equivalent to XY.

Composition and Functions of Chromosomes

Chromosomes are made up of fine filaments of the nucleic acid, DNA. Each filament of DNA is surrounded by a sheath of basic proteins classified as histones. (Figs. 7,8,9). A small amount of acidic protein is also present. The DNA and the protein together constitute the nucleoprotein.

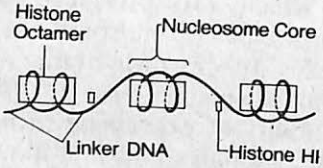


Fig. 7

A schematic representation of nucleosomes. One hundred and forty-five to one hundred and sixty-five base pairs of DNA wound around a histone core, an octamer of H2A, H2B, H3 and H4. Linker DNA of 65 to 35 base pairs connects the adjacent nucleosomes. H1 histone is associated with linker DNA. This arrangement of chromatin material converts the DNA helix of 20A^{0} into a 10nm nucleosome filament.

The complete diploid set of chromosomes, present in every normal somatic cell, contains the cell's hereditary instructions. Each cell in the body, except the germ cells, has an identical genetic complement. The term genome indicates the full set of genes in a gamete, or in a somatic cell, or in an individual. The chromosomal threads bearing these instructions are labelled as genomenata.

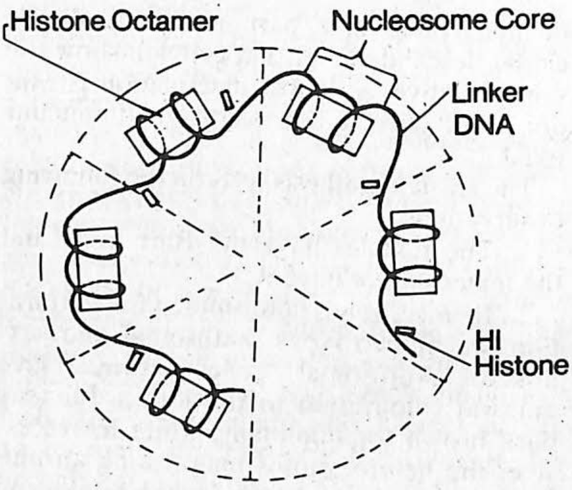


Fig. 8

A schematic model showing arrangement of nucleosomes in transverse section to form 25 nm chromatin fiber. Six nucleosomes are arranged radially in the fibre. Five nucleosomes are shown here. The sixth nucleosome drops down to the next level.

The genes, segments of supercoiled DNA, are thought of as arranged along the chromosome in linear order, each gene having a precise position known as its locus. Alternative forms of a gene which occupy the same locus are called alleles. Any one chromosome bears only one allele at a given locus, though in the population as a whole there may be multiple alleles for that locus. Genes whose loci are in the same chromosome are said to be linked. The function of genes is to direct the synthesis of polypeptides which constitute the proteins. The details of DNA structure and functions are given in Chapter 4.

The nuclear DNA of a cell is packaged in a **hierarchy of supercoils** from the smallest to the largest, in the following manner:

1. **Nucleosome.** The first coil, the nucleosome is a cylinder about 8×11 nm in size. In it, some 146 base pairs of DNA are wound in two turns around a histone core containing two molecules each of the four inner histones, H2A, H2B, H3, and H4. The DNA is thus situated on the outside of the nucleosome. The adjacent nucleosomes are joined to each other by a connecting piece of DNA

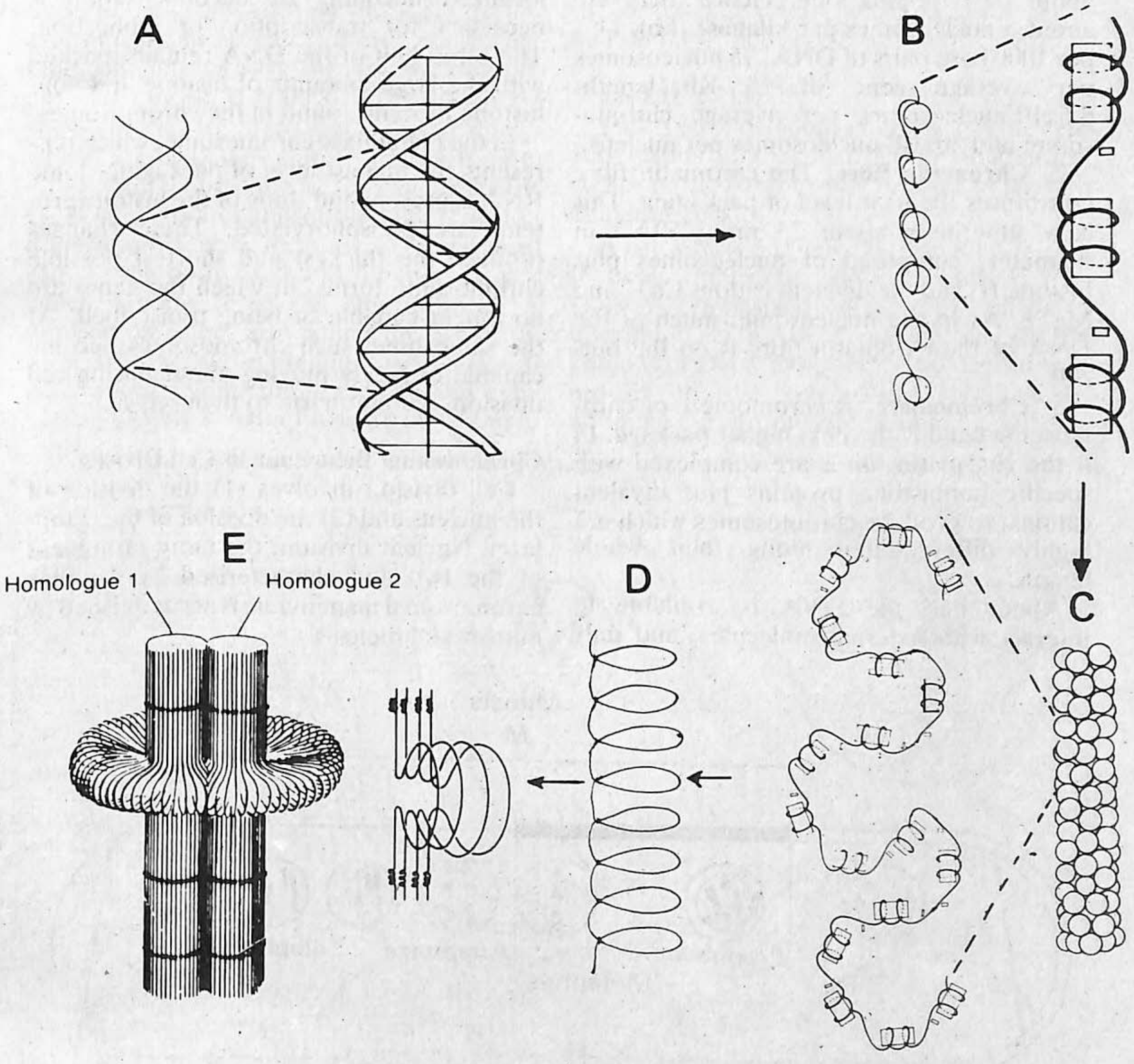


Fig. 9

Levels of packing of chromatin material to form a chromosome.

A. DNA (20 A^0)

B. 10nm nucleosome filament

C. 25nm chromatin fibre

D. Folded loops (0.5 m). Each 25 nm fibre forms a series of loops, each about 0.5 m in length. All the loops are formed along a single axis.

E. Two homologous chromosomes. Each line in the chromosome represents 25nm folded loops fibre shown in D. The chromosomes are showing puffed out regions which develop when chromatin material is actively transcribing.

about 60 base-pair long. Hence there are about 5 nucleosomes per **kilobase (kb),** i.e., per 1000 base pairs of DNA; 75 nucleosomes per average gene of 15 Kb length; 6.5×10^5 nucleosomes per average chromosome; and 30×10^6 nucleosomes per nucleus.

2. **Chromatin fibre.** The chromatin fibre constitutes the next level of packaging. This is a superhelix about 25 nm (250A°) in diameter, composed of nucleosomes plus histone H_1 and the divalent cations Ca^{++} and Mg^{++}. As in the nucleosome, much of the DNA of the chromatin fibre is on the outside.

3. **Chromomere.** A chromomere or chromosome band is the next higher package. In it the chromatin fibres are complexed with specific nonhistone proteins plus divalent cations, to produce chromosomes which are highly differentiated along their whole length.

About half the DNA is available to interact with external molecules, and only localized uncoiling or decondensation is necessary for transcription or replication. The other half of the DNA remains packed with the large amounts of histone and nonhistone proteins found in the chromosomes.

In the metaphase chromosome, which represents the highest level of packaging, some RNA is present and some of the histone proteins are phosphorylated. These changes produce the thickest and shortest possible chromosome forms, in which the genes are no longer capable of being transcribed. At the same time, such chromosomes become capable of freely moving about during cell division, without harm to themselves.

Chromosomal Behaviour in Cell Division

Cell division involves (1) the division of the nucleus and (2) the division of the cytoplasm. Nuclear division, the more prominent of the two and characterised by complex chromosomal maneuvres, is accomplished by mitosis and meiosis.

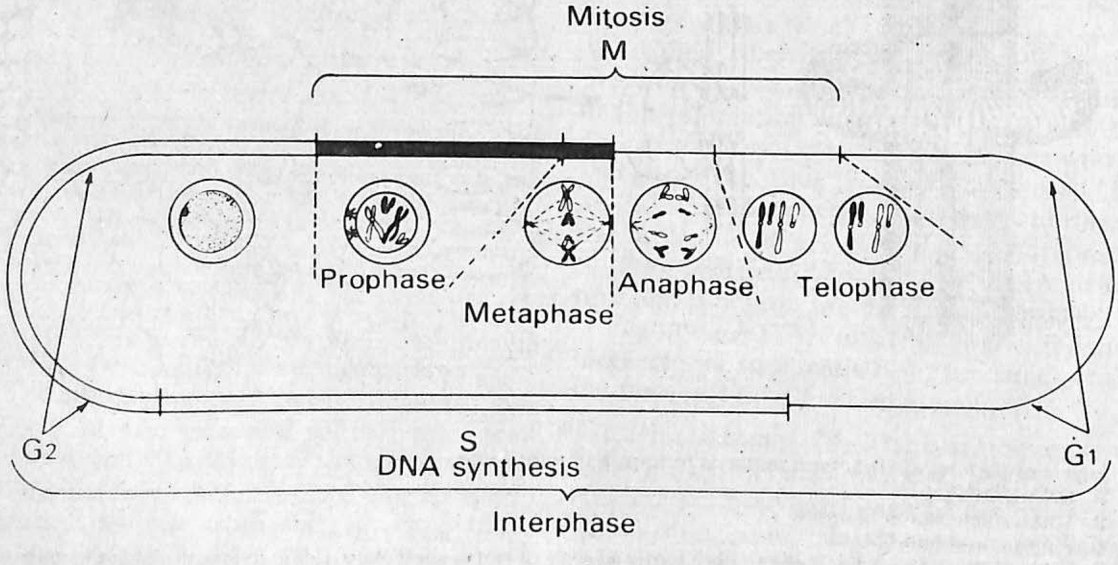


Fig. 10

Diagram illustrating the relative durations of the subdivisions of the mitotic cell cycle. Double lines indicate a double chromatid structure. The relative lengths of the lines would vary from tissue to tissue and from organism to organism. Mitosis and intermitotic phases, G1 (Gap 1), S (Synthesis) and G2 (Gap 2) indicated.

Cells that undergo division regularly, continue to pass consecutively through interphase, mitosis, interphase, mitosis and so on. One complete passage through the interphase and mitosis is termed a cell cycle. As shown in Fig. 10, there are 3 stages through which a cell passes during its interphase. After completing mitosis, the cell enters the G1 (Gap 1) or preduplication stage. The preduplication stage is the state in which most cells of the body exist and function. A cell which is going to divide, enters the DNA duplication stage, also called the S (Synthesis) stage, from the preduplication stage. It takes about 7 hours for all the DNA of a cell to be duplicated. After finishing DNA duplication, a cell passes into the postduplication or G2 (Gap 2) phase, which lasts for about half an hour, before the cell begins mitosis.

Mitosis (Fig. 11)

This type of division occurs in somatic cells and results in the distribution of identical copies of the parent cell's genome to the two daughter cells.

A cell which is not actively dividing is said to be in interphase. As seen earlier, new DNA is synthesized during the S phase of interphase, so that in normal diploid cells the amount of DNA has doubled by the onset of mitosis to the tetraploid DNA value, although the chromosome number is still dip-

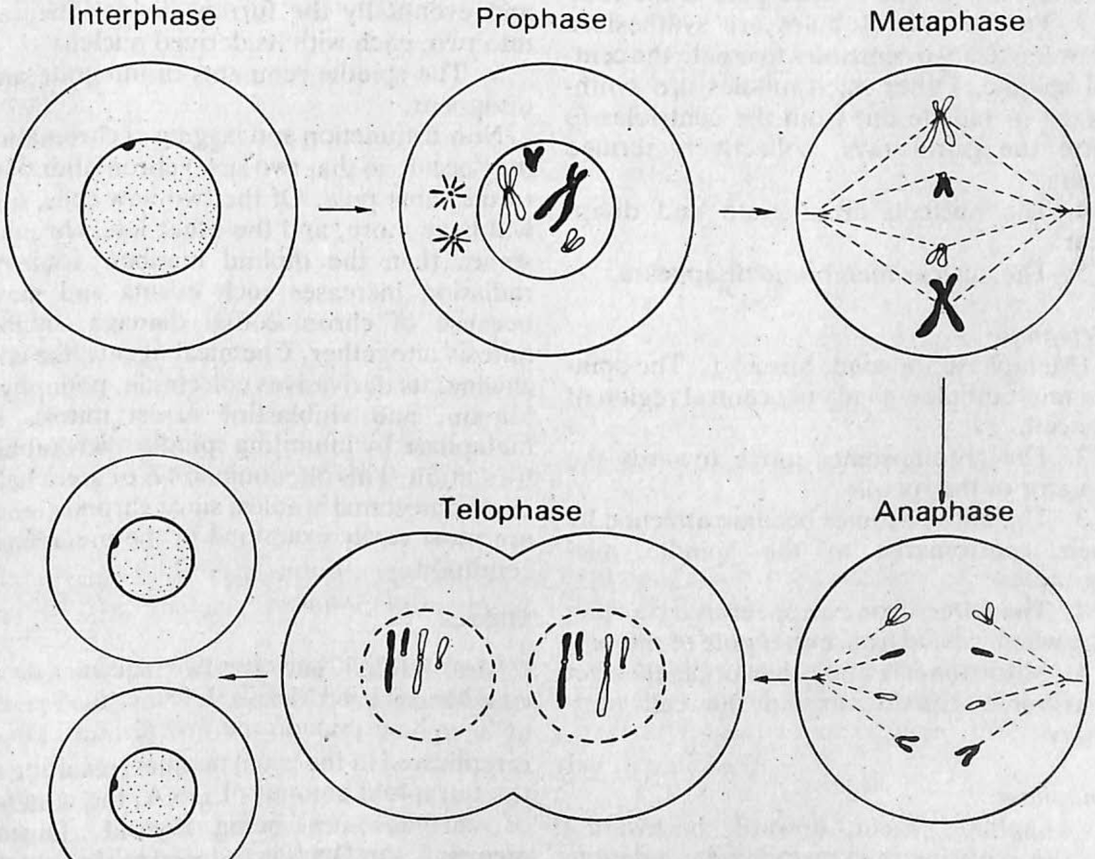


Fig. 11
Schematic representation of various phases of mitosis.

loid. During mitosis, this amount is distributed equally between the two daughter cells, so that DNA quantity and chromosome number become diploid in both the cells. Mitosis is usually described in four stages: prophase, metaphase, anaphase and telophase.

Prophase

(Prophase: beginning; before.) 1. The strands of chromatin get shortened, thickened and become recognizable as chromosomes, each made up of two chromatids joined at the centromere.

2. The centrioles in the cytoplasm separate and move, one to each pole of the cell.

3. Parallel microtubules are synthesized between the two centrioles to create the central spindle. Other microtubules are synthesized to radiate out from the centrioles to form the astral rays, collectively termed asters.

4. The nucleoli disintegrate and disappear.

5. The nuclear membrane disappears.

Metaphase

(Metaphase: beyond; after.) 1. The spindle microtubules invade the central region of the cell.

2. The chromosomes move towards the equator of the spindle.

3. The chromosomes become attached by their centromeres to the spindle microtubules.

4. The chromosomes appear as a star-like ring when viewed from either pole of the cell.

5. Mitochondria and other organelles get distributed equally around the cell periphery.

Anaphase

(Anaphase: again; upward; backward.) 1. The centromere in metaphase is a double structure. It now separates into its two components, each carrying an attached chromatid, so that each of the original chromosomes has, in effect, split into two new chromosomes.

2. The two new chromosomes move apart, one towards each pole of the cell.

3. The chromosomes become grouped at each end of the cell, both groups being diploid in number.

4. Cytoplasmic division begins, by infolding of the cell at the equator.

Telophase

(Telophase: end). 1. The chromosomes re-extend.

2. The nuclear membrane reappears.

3. The nucleoli reappear.

4. The cytoplasmic constriction deepens and eventually the furrow divides the cell into two, each with its derived nucleus.

5. The spindle remnants disintegrate and disappear.

Non-disjunction and lagging of chromatids may occur, so that two sister chromatids pass to the same pole. Of the two new cells, one will have more, and the other less, chromosomes than the diploid number. Ionizing radiation increases such events and may, because of chromosomal damage, inhibit mitosis altogether. Chemical agents like colchicine, its derivatives colcemide, podophyllotoxin, and vinblastine arrest mitosis in metaphase by inhibiting spindle microtubule formation. This phenomenon is of great help in chromosomal studies, since chromosomes are most easily examined in the metaphase condition.

Meiosis

Meiosis (Fig.12) involves two sequential divisions: Meiosis I and Meiosis II. During the S phase of interphase prior to the first division, DNA is replicated in the usual manner, resulting in the tetraploid amount of DNA, the number of chromosomes being diploid. During meiosis I, the DNA is reduced to the diploid amount in each chromosome, and the chromosome number is halved to the haploid. In meiosis II, the DNA in each new

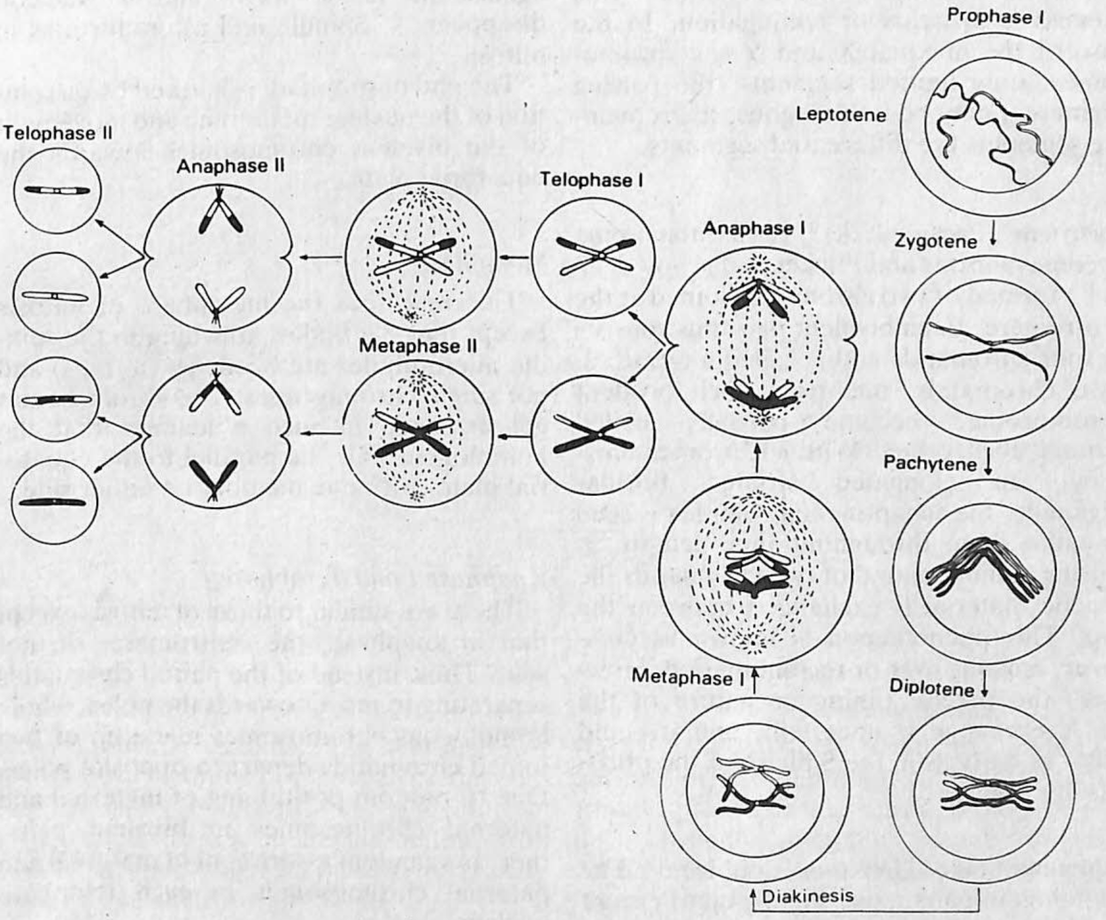


Fig. 12

Schematic representation of various phases of meiosis I and II. The four gametes formed are indicated.

daughter cell is reduced to the haploid amount, the chromosome number remaining haploid.

Meiosis I and II each, like mitosis, can be divided into prophase, metaphase, anaphase and telophase.

Meiosis I

Prophase I. This is a long and complex phase which differs markedly from mitotic prophase. It is divided into leptotene stage, zygotene stage, pachytene stage, diplotene stage and diakinesis.

Leptotene stage. (Leptos: slender; tainia: ribbon.) 1. The chromosomes become visible as individual threads. 2. One end of the threads is seen to be attached to the nuclear membrane. 3. Chromosomal threads show characteristic beads (chromomeres) throughout their length.

Zygotene stage. (Zygon: yoke; synaptic: joined.) 1. Chromosomes come together side by side in homologous pairs. 2. The homologous chromosomes pair, point for point, so that corresponding regions lie in contact. This process is synapsis or conjugation. In the case of the unequal X and Y sex chromosomes, only limited segments (the pairing segments) may be homologous; the remaining segments are differential segments.

Pachytene. (Pachys: thick) 1. Each chromosome becomes shorter and thicker and is now seen to be formed of two chromatids joined at the centromere. Each bivalent pair thus consists of four chromatids and is called a tetrad. 2. Two chromatids, one from each bivalent chromosome, become partially coiled around each other. With electron microscopy, an elongated striated fibrillar organelle, the synaptinemal complex is seen to unite them throughout their length. 3. During coiling around of two chromatids the genetic material is exchanged between the two. This phenomenon is known as crossover, **crossing over** or recombination. However, the precise timing or nature of the DNA exchange is uncertain, and it could occur as early as in the S phase of the previous interphase.

Diplotene stage. (Diplous: double) 1. The homologous pairs move slightly apart except at the points seen as chaismata, where crossing over has occurred. Sometimes the chiasmata move towards the end of the chromatids. Normally at least one chiasma forms between each pair of homologous chromosomes, and up to five have been observed. 2. The chiasmata break.

In human meiosis, primary oocytes become diplotene by the fifth month *in utero*, and remain in this state till the period prior to ovulation.

Diakinesis. (Dia: across+Kinesis=movement, motion.) 1. The remaining chiasmata finally break. 2. The chromosomes, still bivalents, become shorter and thicker. 3. The bivalents move away from each other and spread out against the nuclear membrane. 4. Nucleoli disappear. 5. Spindle and asters form as in mitosis.

The end of prophase is marked by dissolution of the nuclear membrane and movement of the bivalent chromosomes towards the equatorial plate.

Metaphase I

This resembes the metaphase of mitosis except that the bodies attaching to the spindle microtubules are bivalents (tetrads) and not single chromosomes. The chromosomes get arranged in such a manner that the homologous pairs lie parallel to the equatorial plate, with one member on either side.

Anaphase I and Telophase I

These are similar to those of mitosis except that in anaphase, the centromeres do not split. Thus, instead of the paired chromatids separating to move towards the poles, whole homologous chromosomes made up of two joined chromatids depart to opposite poles. Due to random positioning of maternal and paternal chromosomes in bivalent pairs, there is a random assortment of maternal and paternal chromosomes in each telophase nucleus.

Cytoplasmic division occurs as in mitosis to produce two new daughter cells.

Meiosis II

This takes place after meiosis I following a brief interval during which *no DNA synthesis occurs.* The second meiotic division is similar to mitosis with the exception that the separating chromatids in the anaphase are genetically dissimilar. Cytoplasmic division follows and thus a total of four cells results from meiosis I and II.

If cytoplasmic division fails to occur during meiosis I, gametes with the diploid number of chromosomes result, and thus following fertilization a triploid zygote results. Other

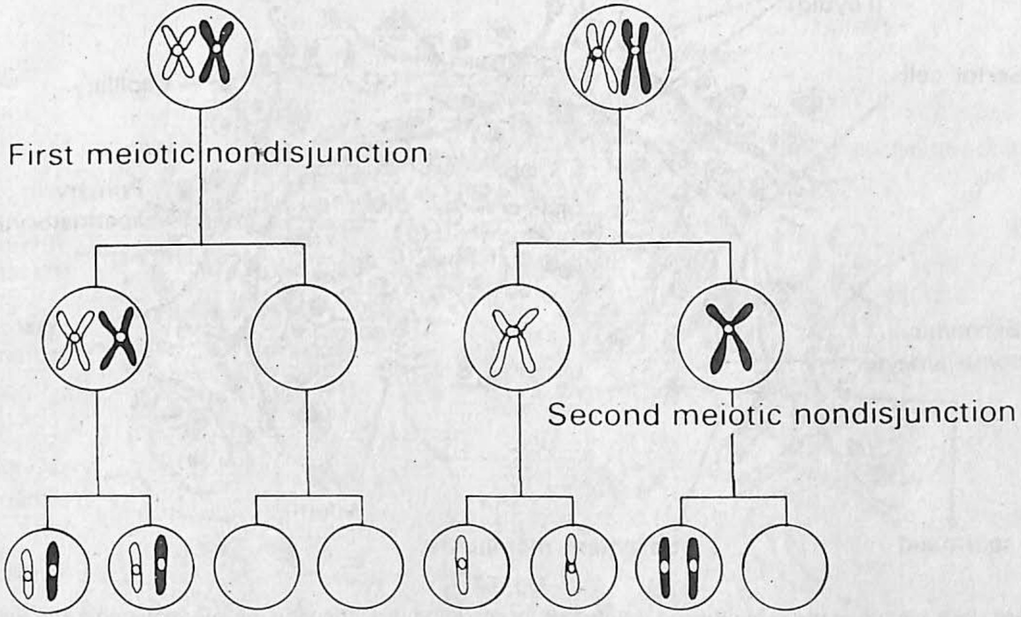


Fig. 13

Scheme to show nondisjunction in meiosis I and II and the resulting abnormal gametes.

abnormalities are produced by **non-disjunction** of chromosomes (Fig.10), or by the lagging of individual chromosomes, during anaphase. There is some indication that with increasing maternal age, there is increased frequency of such abnormalities.

Human Spermatogenesis

Male gonad. The testis or the male gonad, lies in the scrotum covered by a serous sac, the **tunica vaginalis**. Its dense fibrous capsule called the **tunica albuginea** is thickened posteriorly to form the **mediastinum testis** which sends in fibrous partitions to divide the testis into about 250 lobes or lobules. Each lobe contains from one to four highly convoluted **seminiferous tubules** varying from 30 to 70 cm in length and 150 to 250 μ in diameter, as also the interstitial endocrine tissue. (Fig. 14) The stratified epithelium of the tubules produces the male gametes, the **spermatozoa;** and the **interstitial cells of Leydig** produce the male hormone **testosterone.** The sperms are conducted to the prostatic part of the urethra via a tortuous course from the convoluted portion of the seminiferous tubule to its straight part, and then through the rete testis, efferent ductules, canal of the epididymis, ductus deferens and finally the ejaculatory duct.

A functionally active, postpubertal seminiferous tubule is lined along its length and around its circumference, by two distinct cell types.

1. Single-layered and scattered sustentacular **cells of Sertoli** resting on the basement membrane of the tubule and
2. Multiple rows of spermatogenic or germ cells at varying stages of maturation. The basal row of these cells also rests on the basement membrane.

Each Sertoli cell is an elongated pyramidal cell with its base attached to the basement membrane. Its irregular apical surface faces the lumen, and its deeply recessed sides are moulded by the maturing spermatids of varying shapes. The developing germ cells get protection, mechanical support and some

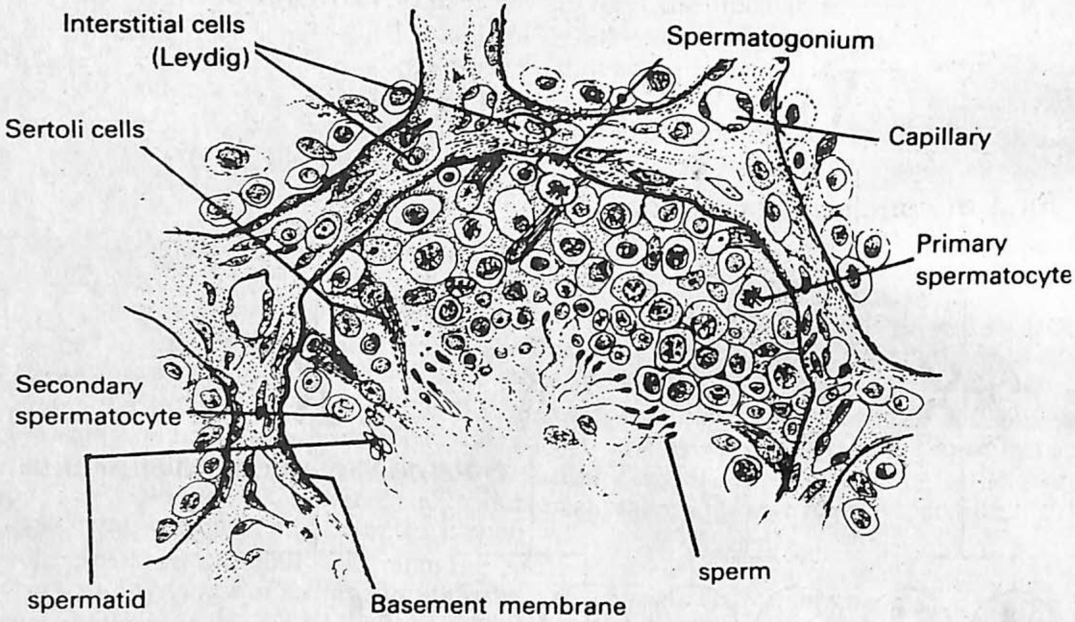


Fig. 14

Transverse section of the seminiferous tubule and its surroundings, showing the spermatogenic and Sertoli cells and the endocrine interstitial cells.

nutrition from the Sertoli cells. The Sertoli cells play an active role in the liberation of mature sperms, as is suggested by their irregular sides and apices, their notably large surface area and their everchanging shapes and contours. Sertoli cells have not been seen to divide in a mature testis; and unlike the germ cells they are highly resistant to a number of cytotoxic agents, ionizing radiation and heat.

The spermatogenic cells from the periphery of the tubule to its centre include spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. These structurally distinct cell types are successive stages in the differentiation of germ cells (Fig. 15).

Spermatogenesis

The continuous series of events by which spermatogonia are changed to sperms is called spermatogenesis. It is divisible into three phases

1. Spermatocytogenesis
2. Meiosis
3. Spermiogenesis

1. **Spermatocytogenesis**

- Spermatogonia multiply by mitotic division, thus assuring a continuous supply of spermatogonia
- Some spermatogonia modify themselves, increase in size and push themselves towards the lumen of the tubule to become primary spermatocytes
- Primary spermatocytes contain enough nutrient material to provide for succeeding stages of maturation
- All but the earliest spermatogonia remain interconnected by cytoplasmic bridges with the result that one spermatogonium gives rise to a large cluster of interconnected spermatids (Fig. 16).

2. **Meiosis**

It takes place in two stages.

Stage I

- Primary spermatocytes undergo duplication of their chromosomal material.

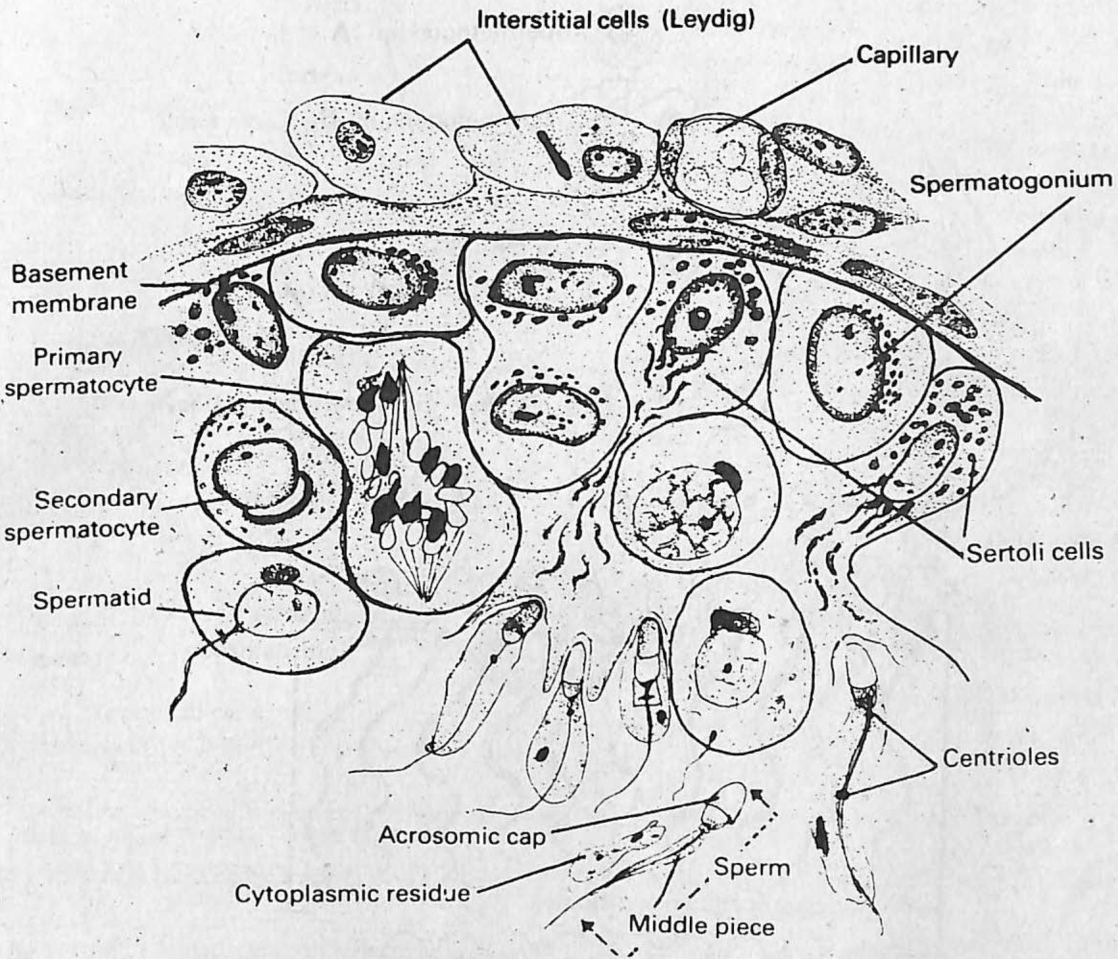


Fig. 15

Transverse section of a seminiferous tubule seen under high magnification, showing cellular detail.

- Primary spermatocytes enter a long prophase lasting for 22 days
- During the prophase the homologous chromosomes form pairs and exchange parts of their segments — "crossing over."
- During anaphase, the centromeres do not split and thereby each member of a homologous pair of chromosomes made up of two joint chromatids, departs to opposite poles.
- The end result is a secondary spermatocyte, slightly smaller than the primary, having only half the number of chromosomes, some of which are paternal and some maternal in origin.

Stage II

- After a brief prophase II, the secondary spermatocytes — haploid cells — enter metaphase II followed by anaphase II.
- During anaphase II, the centromeres holding the chromatids together in each chromosome, divide to separate these chromatids.
- One set of chromatids passes to each new daughter cell, which is now called a spermatid.
- A spermatid contains half the species

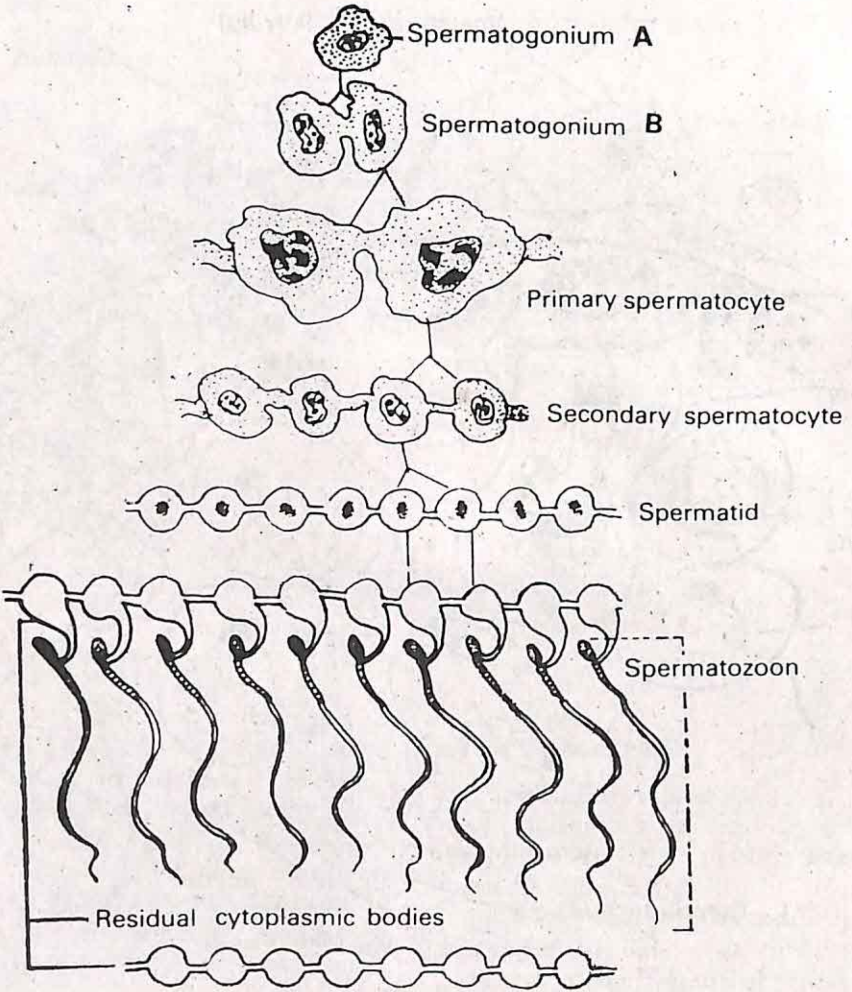


Fig. 16

Stages in spermatogenesis. Male germ cells arise as a clone of cytoplasmically interconnected cells.

number of chromosomes and half the genetic material of a secondary spermatocyte.

To summarize, each spermatogonium gives rise to two interconnected primary spermatocytes, which in turn divide to give four interconnected secondary spermatocytes which again divide to form eight interconnected spermatids.

3. Spermiogenesis

This is a process by which cytological changes transform inert spermatids into actively motile sperms.

The changes are:

- progressive condensation of the nucleus
- transformation of Golgi material into the acrosome
- origin of the axoneme from the distal centriole
- formation of mitochondrial and fibrous sheaths
- elimination of the redundant cytoplasm of the spermatid as the Residual Body of Regnaud.

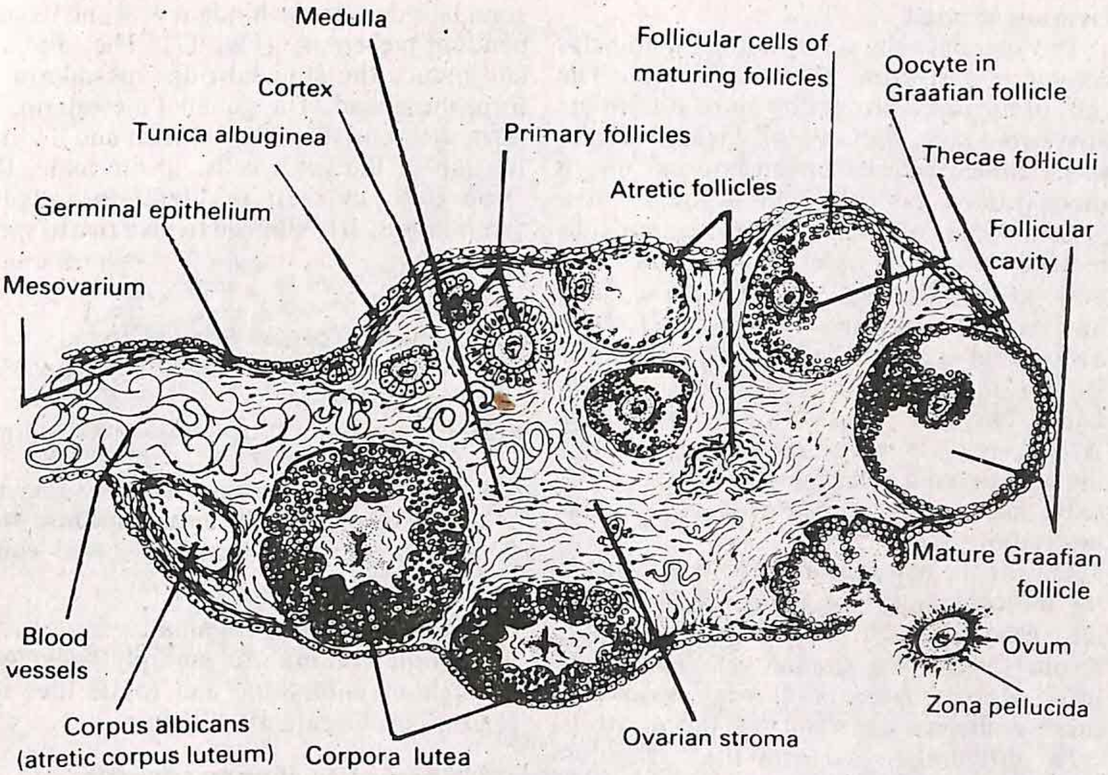


Fig. 17

Schematic panoramic section of the ovary, showing ovarian follicles in various stages of maturation. A ruptured follicle and two corpora lutea also shown.

THE FEMALE GENITAL SYSTEM

The female genital system, essentially, consists of the two ovaries, the two uterine tubes, the uterus and the vagina. It shows cyclical changes repeating about every four weeks, starting at puberty and ceasing at menopause.

The Female Gonad

The ovary or the female gonad (Fig.17) is a solid organ situated in the pelvic cavity along its side wall. It has a peripheral **cortex** and a central **medulla.** During the child-bearing period, i.e. from puberty to menopause, the cortex occupies most of the bulk of the ovary. It consists of:

1. A highly cellular, embryonic connective tissue stroma and
2. The germ cells or oogonia and their enclosing follicular cells, together constituting the ovarian follicles, embedded in the stroma.

A thin fibrous sheath, the **tunica albuginea,** covers the cortex. The tunica is lined on its exterior by cubical cells of the **germinal epithelium.** The medulla consists of vascular connective tissue, lymphatics and some vestiges of the "testicular", mesonephric tubular and duct systems.

Ovary as an endocrine gland

The cells derived from

1. the ovarian stroma and
2. the ovarian follicles

are both endocrine in function.

Ovarian stroma

The stromal cells surrounding the follicles constitute a covering, the theca folliculi. The cells of the theca proliferate and differentiate into two zones, an internal, highly vascular **theca (tunica) interna** and an external fibrous **theca (tunica) externa.** Even in the pre-adolescent ovary, when no follicles become fully mature, they continue to grow and later undergo atresia, and the cells of the theca interna proliferate and differentiate to form a thick region of lipid-laden cells — the interstitial gland. The role of these cells is uncertain. They may produce a little oestrogen. After puberty however, these cells constitute the well-defined thecal gland which is known to be the chief source of oestrogens — mainly oestradiol.

During the maturation of ovarian follicles. the follicular cells multiply and differentiate into granulosa cells of the stratum granulosum (membrana granulosa). These also produce oestrogens. Following ovulation, these granulosa cells and the theca interna cells differentiate to form the "granulosa lutein cells" and the "theca lutein cells" respectively, of the **corpus luteum,** which produce progesterone. Some androgens or male hormones are also produced by the ovarian stromal cells. Another hormone called **relaxin**, which facilitates parturition or delivery, by helping to dilate the cervix, is also produced from some unspecified stromal cells.

The Ovarian Follicles

The ovarian follicles are concerned with oogenesis, the process by which mature ova or female gametes are produced. Initially, each follicle consists of an oogonium — or germ cell — the precursor of an ovum, and its surrounding flattened follicular cells. This is known as a **primordial follicle.** The oogonia are derived from the primordial germ cells which appear by the end of the third week of gestation, at the caudal end of the yolk sac. Probably entodermal in origin, these cells migrate to reach the mesoderm of the gonadal ridge via the hindgut wall and its suspending mesentery (Fig. 17). They initiate and induce the gonadal ridge mesoderm to form the gonad. The gonadal mesoderm, in turn, is essential for the survival and multiplication of the germ cells. In the male, the same cells, by their proliferation and differentiation, are believed to give rise to spermatogonia.

Oogenesis

Oogenesis occurs in three phases:

1. Multiplication of oogonia by repeated mitotic divisions.
2. Enlargement of some oogonia to form primary oocytes
3. Maturation of some of these oocytes into ova, through the **two meiotic divisions — reductional** (first) and **equational** (second).

1. Multiplication of oogonia

Oogonia continue to multiply by mitosis throughout embryonic and foetal life, but cease to proliferate after birth.

2. Formation of primary oocytes

Some oogonia continue to enlarge and differentiate into primary oocytes, which are later surrounded by a layer of flat stromal (follicular) cells to form primordial ovarian follicles. At birth, each ovary contains about two million of these. The primary oocytes show nuclear changes preparatory to meiosis-I. Most of these remain in this state till puberty, and continue to degenerate, so that at puberty they number about 40,000.

3. Maturation of ovarian follicles (Fig. 18)

The primordial follicles, and the primary oocytes they contain, continue to grow during foetal life, and can come to full maturation only after puberty. During maturation they pass through the following stages:

i. Primary follicles: The follicular cells become columnar. The oocytes grow and secrete a thick, pale, structureless covering, the zona pellucida.

ii. Secondary follicles: The follicular

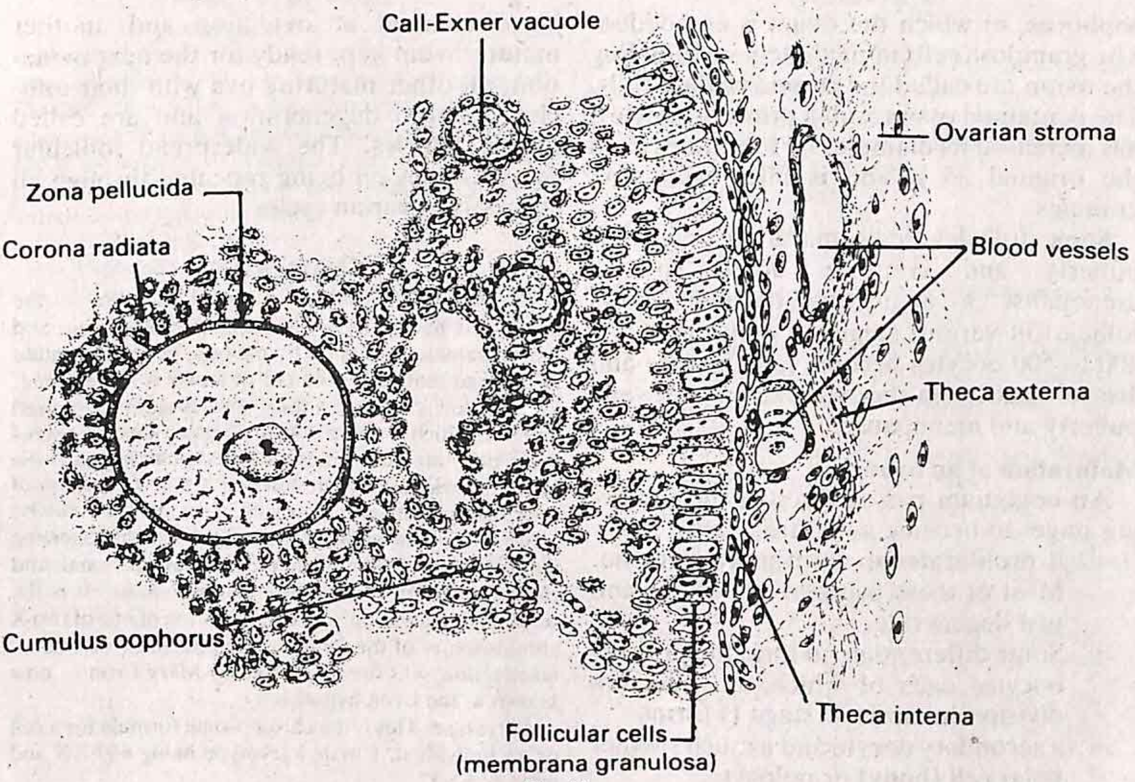


Fig. 18
A section through a Graafian follicle.

epithelium proliferates to become stratified and is called the membrana granulosa. The zona pellucida is fully formed. It is, in fact, the joint product of the oocyte and the covering follicular cells. In the region of the zona, the microvilli of the oocyte plasma membrane intermingle with the inward projecting cytoplasmic processes of the innermost layer of follicular cells. Thus, the follicular cells can convey nutrition to a large surface area of the oocyte. By six months IUL, many primary and secondary follicles are present. By the eighth foetal month, most of the primary oocytes have reached the diplotene stage of the prophase of meiosis-I, and remain in this stage till they fully mature prior to ovulation.

iii. Vesicular or Graafian follicles: While most of the follicles degenerate to become atretic follicles, some of the secondary follicles enlarge and develop a crescentic follicular cavity containing fluid — the liquor folliculi, within the mass of granulosa cells. These are then called vesicular or Graafian follicles. At ovulation, one of these follicles matures and ruptures to release the contained ovum.

Mature vesicular follicle

The mature follicle is a fibrocellular sac, which has an eccentric cavity, the antrum folliculi, filled with fluid, the liquor folliculi. The wall of the follicle shows from the centre to the periphery:

i. the cells of the stratum granulosum
ii. the cells of the vascular theca interna and
iii. the fibrous theca externa.

On one aspect of the follicular wall, there is a hillock of granulosa cells, the **cumulus**

oophorus, in which the ovum is embedded. The granulosa cells immediately surrounding the ovum are called the **corona radiata** cells. The contained ovum (still a primary oocyte), has increased its diameter by four times from the original 35 μ and is filled with yolk granules.

Some follicles begin maturing only after puberty and continue doing so till menopause. So, at any time, the ovary shows follicles in various stages of maturity. Only 400 to 500 oocytes become fully mature and are released from the two ovaries, between puberty and menopause.

Maturation of an ovum

An oogonium passes through the following stages to become a mature ovum:

i. it proliferates to form more oogonia. Most of these proceed no further and just degenerate.
ii. Some differentiate to form the primary oocytes each of which by reduction division (meiosis — stage I) forms
iii. a secondary oocyte and a much smaller polar cell (body) or polocyte.
iv. The secondary oocyte undergoes the second maturation division (meiosis — stage II) to form the ootid or mature ovum and a much smaller second polar cell. Meiosis — stage II is only completed at fertilization.

Ovarian cycle

The ovary undergoes cyclic changes which are repeated every four weeks, starting at puberty and ending at menopause. The ovarian cycle consists of:

i. Proliferation and maturation of oocytes.
ii. Discharge of an ovum at ovulation.
iii. Repeated formation of corpus luteum following each ovulation and the accompanying changes occurring in the ovarian stroma in general, and the thecal gland in particular.

Several ovarian follicles begin maturing at each cycle. Except the fully mature ovum to be discharged at ovulation and another mature ovum kept ready for the next ovulation, all other maturing ova with their follicles undergo degeneration and are called **atretic follicles.** The widespread follicular atresia keeps on being repeated through all successive ovarian cycles.

SUMMARY

Chromosomes: Organelles found in pairs in the eukaryotic nuclei, have a species-specific number and during gamete formation by meiosis, get redistributed in them so that each member of a pair is represented. Fertilization restores the species-specific paired number, which for man is 46 or 23 pairs. Twenty-two of these pairs are autosomes and the remaining pair, the sex chromosomes, are designated X and Y, because of their dissimilarity and their role in sex determination.

Sex chromatin or Barr body: This is a characteristic feature of interphase nuclei of all female mammals and can be used for 'nuclear sex determination.' It is the result of 'inactivation' and condensation of one of two X chromosomes of the female. The possibility of 'one X-inactivation' was first suggested by Mary Lyon — now known as the Lyon hypothesis.

Karyotype: This is the chromosome formula for a cell or an individual, female karyotype being 44+XX and male 44+XY.

Classification of chromosomes: They are classified and numbered on the basis of their decreasing length and position of their centromeres.

Study of chromosomes: They can be studied directly by techniques of tissue-culture and variety of staining-techniques, chief of which are quinacrine fluorescent and Giemsa methods. With these, each chromosome shows a characteristic 'banding pattern'. Elaborate nomenclature has been established to describe chromosomal fractions and subfractions.

Chromosome structure: Each chromosome is a supercoiled package of DNA and several proteins. Progressive anatomization of a chromosome reveals it to be made of chromomeres, each chromomere made up of coils of chromatin fibre, each chromatin fibre is made of smaller units, the nucleosomes, each nucleosome comprising of DNA and histones.

Chromosomes are best seen when they are maximally coiled. This takes place during metaphase of mitosis and meiosis. Metaphase chromosomes are best suited for detailed karyotyping.

Mitosis: All cells except maturing gametes increase in number by means of mitosis. Following doubling or replication of DNA, a replicating cell passes through following continuous, overlapping phases:

1. Prophase 2. Metaphase
3. Anaphase and 4. Telophase.

Meiosis: This occurs only in gonads during gametogenesis, in two steps, meiosis I and meiosis II. The two steps follow in quick succession without an intervening DNA replication phase. Stages in meiosis I and II are the same as in mitosis, except that the prophase of meiosis I is long-drawn and complex. It is characterized by the following stages:

1. Leptotene 2. Zygotene 3. Pachytene
4. Diplotene 5. Diakinesis.

At the end of meiosis I, each of the two daughter cells carries 23 bivalent (two sister chromatids) chromosomes.

Meiosis II is an equating division, wherein 23 bivalents give rise to 46 chromosomes due to the longitudinal splitting of each centromere, and each sister chromatid becoming an independent chromosome and these 46 get shared as 23 each in the resulting daughter cells or gametes.

In spermatogenesis, all resulting gametes are functional. In oogenesis, only one out of four daughter cells is functional, the other three being polar bodies or cells.

QUESTIONS

1. What are chromosomes? Give an account of their structural organization.
2. Define an allele, giving examples.
3. What is a karyotype? Mention, briefly, the technique for karyotyping.
4. Write a note on

   Sex chromatin
   Nucleosome
5. Distinguish clearly, between euchromatin and heterochromatin.
6. Describe mitosis, explaining its significance.
7. Describe meiosis, explaining its significance. State how it differs from mitosis. What is "crossing over"?
8. Write a note on the X chromosome. Mention some important X-linked traits.
9. Write a note on the Y chromosome.
10. Classify chromosomes.
11. Describe the chromosomal banding techniques.
12. Discuss the Lyon hypothesis.

3 Cytogenetics - Abnormal Chromosomes

INTRODUCTION

Abnormal chromosomes are the vehicles of inherited abnormalities. The abnormalities may be either numerical or structural or both, and may occur during mitosis or meiosis. Structural aberrations result from single or multiple breaks along the chromosomal length. The broken fragments are then either destroyed (deleted) or rearranged in various ways or shifted (translocated) to other chromosomes. Such "chromosomal mutations" may occur spontaneously i.e, without an apparent cause, or be triggered by external agents e.g., ionizing radiations or some drugs.

It is only through the abnormal gametes formed at meiosis that chromosomal anomalies can be passed on from one generation to the next.

The usual forms which occur as structural abnormalities are **deletions, duplications, translocations, inversions, isochromosomes** and **ring chromosomes.**

Abnormal chromosome numbers result almost invariably from the phenomenon of non-disjunction during the first and/or the second meiotic divisions. Common examples of such anomalies include **Down's syndrome, Turner's syndrome** and **Klinefelter's syndrome.**

The causes of chromosomal aberrations are not precisely known. However, it is observed that certain factors predispose to such aberrations. These include ionizing radiations, viruses, chemical carcinogens, late maternal or paternal age, and possibly a few specific genes themselves.

* * *

Abnormal Chromosomes

The normal functioning of the genetic system of an individual is dependent upon the constancy of hereditary material carried in the chromosomes. Sometimes changes do occur in chromosomes, which are brought about spontaneously or by external agents such as X-rays. Chromosomal aberrations are either numerical or structural, and may affect either autosomes, sex chromosomes, or both, in the same karyotype.

The vast majority of chromosomal abnormalities occurring at conception are lethal, either in early pregnancy or later, and only a small proportion of all such abnormalities get exhibited as live births. This is an example of natural selection at work, mediating quality control in human reproduction.

The abnormalities, in structure or number of chromosomes, can arise at any time in any cell of the body, during one's lifetime. The abnormality affecting the somatic cell has no consequences on the progeny of an individual. However, if the abnormality affects gonadal cells or arises during gametogenesis, it will be passed on to the progeny via the gametes. The zygote resulting from such a gamete will pass on the abnormality to all the cells of the body. Such a person becomes a **carrier** of the anomaly. In general, any person who has a chromosomal abnormality within his gonadal cells, which will have an effect on gametogenesis, is referred to as a carrier of chromosomal abnormality.

Structural Abnormalities

Changes in the arrangement of the chromosomal material generally occur following breaks in the chromosome. The breaks are produced either spontaneously or are induced by such external factors as radiation, chemicals or viruses. There is some evidence that chromosomal breaks occur more frequently than chromosomal aberrations; this means that broken chromosomes often unite according to the original configuration, leaving behind no trace of the break.

Deficiency or Deletion (Fig. 19)

Deletion is the loss of a portion of a chromosome. If there is a single break in the chromosome, only the terminal portion of the chromosome is deleted. If there are two breaks in a chromosome, the interstitial portion between the two breaks is deleted, followed by the union of the broken ends of the chromosome. The deleted portion, if it lacks a centromere, is an acentric fragment, which, because it has no centromere, fails to move on the spindle, and is eventually lost during a subsequent cell division unless the deleted fragment gets attached to the same or another chromosome. Deletion of part of a chromosome is often referred to as partial monosomy.

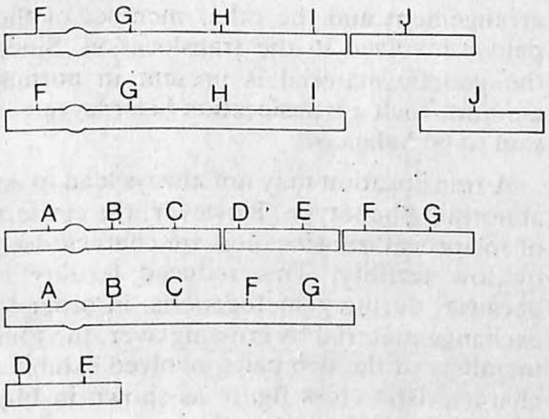


Fig. 19

Deletion, terminal and interstitial. Chromosomes with sites of break indicated. Deletion of J (terminal) and DE (interstitial) are shown.

Deletions have been indicated by a minus sign (−) after the arm of the chromosome involved. 5p−, for example, means that the short arm of 5 is shorter than usual. It is now indicated after the Paris Conference (1971), by the addition of "del" before the chromosome number, to distinguish a simple deletion from other instances where the shortening was part of other aberrations, such as translocation or pericentric inversions. The full karyotype of the above example may be stated as 46, XY, del (5p) without the need to use a minus sign. If the break-point bands are known, they are added in another parenthesis; thus 46XY, del(5) (p13) would indicate the terminal deletion of the segment, distal to the 5p 13 band.

The consequences of a chromosomal deficiency depend on its length and the role of the genetic material involved. In Drosophila, where minute deficiencies can be detected and measured accurately, deletion in some areas of even a single chromomere may be lethal. Whereas, for other areas, loss of even fifty chromomeres may be compatible with life, if the organism is heterozygous for that deficiency. In a heterozygous deficiency, one chromosome is normal, but its homologue is deficient. By and large, experiments on animals with a homozygous deficiency, indicate that they usually do not survive to an adult stage because a complete set of genes is missing. This suggests that most genes are indispensable, at least in a single dose, for the development of a viable organism. Deletions are important in investigations of gene location for determination of the presence and position of unmatched genes.

The common example of deletion in humans is the *cri du chat* syndrome, in which part of the short arm of chromosome 5 is deleted.

In man, Turner's syndrome is an XO condition resulting from the deletion of a whole chromosome. This is the most evident and the most frequent chromosomal deficiency in man, compatible with life and related to the X chromosome.

Ring Chromosome

A ring chromosome is a type of deletion chromosome. If two breaks occur in a chromosome at its two ends, and both the ends get lost, the two sticky broken ends of the chromosome unite to form a ring (Fig. 20). Ring chromosomes are basically deficiencies and therefore the consequences will depend on the length and the role of the genetic material lost. In addition, the carrier of such a chromosomal aberration, will produce abnormal gametes as shown in Fig. 21. Crossing-over between a ring chromosome and its homologous chromosome produces a dicentric chromosome. Moreover, during mitosis, ring chromosomes often get interlocked, instead of there being free rings with one centromere each.

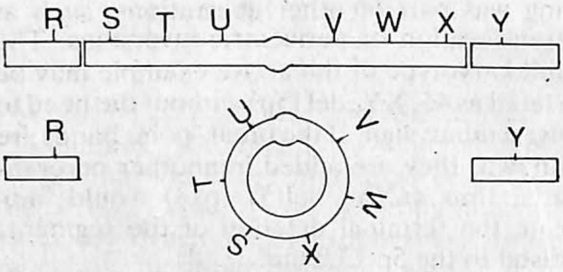


Fig. 20
Ring chromosome formation follows two terminal breaks, fragments R and Y get deleted.

Ring chromosomes have now been found in every chromosome group. Many of them have not been associated with any consistent syndrome, except that mental retardation is a consistent feature. Ring X chromosomes have also been seen. The carriers of ring X chromosome may show features of Turner's syndrome and are quite often sex chromatin positive.

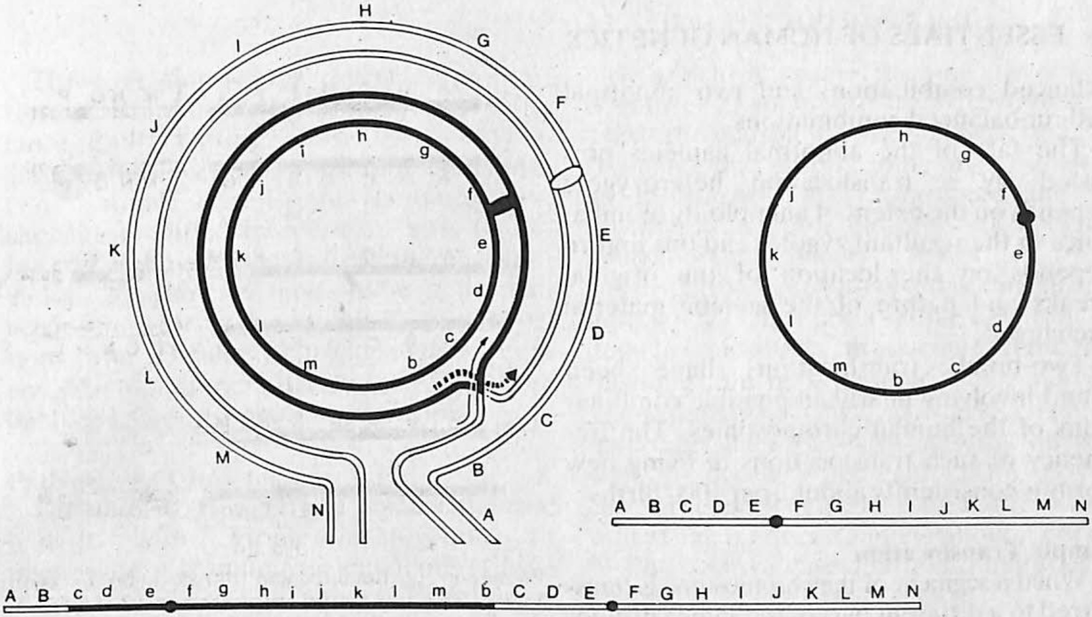
Translocation

The most frequently observed abnormality in the structure of a chromosome is a break at two places. If the two breaks are on nonhomologous chromosomes, the chromosomes may exchange pieces, forming a **reciprocal translocation.** When the two homologous chromosomes are involved, the exchanged segments are nonhomologous.

Reciprocal translocation is the most commonly encountered aberration in well-studied organisms with more than one chromosome pair. Since breakages are usually random in these cases, the chances are greater that the two breaks will be on different chromosomes rather than on the same chromosome. It is not surprising therefore that the two-break reciprocal-translocation is the most commonly encountered structural abnormality in human chromosomal analyses.

Reciprocal translocations may be **homozygous** when both members of a chromosomal pair have exchanged segments with another pair. If only one member each of both the pairs has exchanged segments, then it is a **heterozygous reciprocal translocation.** All human carriers of reciprocal translocations have proved to be heterozygous for the aberration. As shown in Fig. 22 one member of each pair maintains the normal arrangement and the other member of the pair is involved in the translocation. Since the genetic material is present in normal amount, such a **translocation heterozygote** is said to be **balanced**.

A translocation may not always lead to an abnormal phenotype. However, the carriers of reciprocal translocation are characterised by low fertility. This reduced fertility is because, during gametogenesis, in order to exchange material by crossing over, the four members of the two pairs involved exhibit a characteristic cross figure as shown in Fig. 23. This tetrad reaches the equator of the spindle during the first meiotic division, oriented in different ways in relation to the poles. As shown in Fig. 23, the distribution of segregation is random, and at least two-thirds of the gametes of the carriers of a reciprocal translocation would have an unbalanced chromosomal complement. The formation of gametes would be in the proportion of one normal, one abnormal with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A B c d e f g h i j k l m b C D E F G H I J K L M N

Fig. 21

Crossing over during meiosis I in ring chromosome. Note that one abnormal dicentric chromosome results. The sister chromatids of ring chromosome and its homologous chromosome remain unchanged as they are not involved in crossing over. The result would be 3 abnormal gametes, one bearing a dicentric chromosome, the other bearing no chromosome and the third one a ring chromosome; as also one normal gamete bearing a normal homologous chromosome.

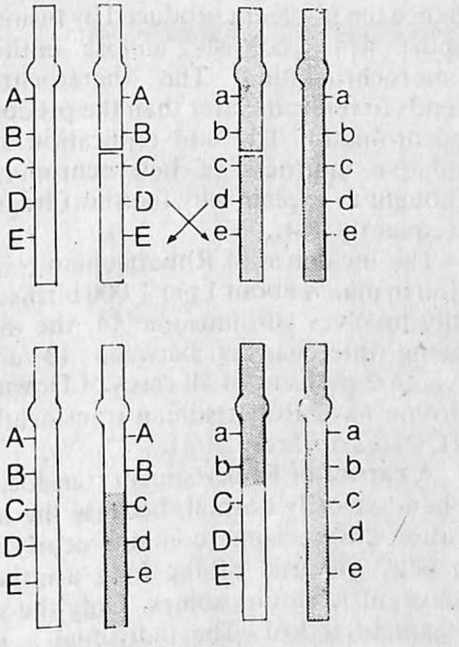

Fig. 22

Reciprocal translocation. Two pairs of nonhomologous chromosomes, with two sites of break in one member of each pair, is shown above and reciprocal interchange of fragments between only one member of each pair (heterozygous) is shown below.

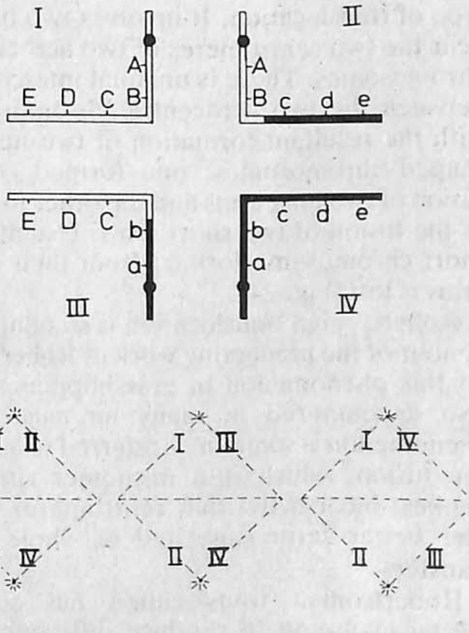

Fig. 23

Gametogenesis in reciprocal translocation showing a cross configuration during crossing over. Abnormal gametes are (I II), (III IV), (I, III), (II IV). Normal gamete is (I IV). Abnormal but balanced is (II III). These are chance results due to random assortment.

balanced combination, and two abnormal with unbalanced combinations.

The fate of the abnormal gametes produced by a translocation heterozygote depends on the extent of aneuploidy or imbalance in the resultant zygote; and this in turn depends on the location of the original breaks and nature of the genetic material exchanged.

Two-break translocations have been found involving nearly all possible combinations of the human chromosomes. The frequency of such translocations in living new borns is consistently about 2 per 1000 births.

Simple Translocation

When a segment of the chromosome is transferred to a different part of the same chromosome or to another chromosome, it is referred to as simple translocation. This is an extremely rare event, if it occurs at all.

Robertsonian Translocation

Robertsonian translocation is a special type of translocation. It involves two breaks near the two centromeres of two acrocentric chromosomes. There is unequal interchange between the two acrocentric chromosomes with the resultant formation of two new V-shaped chromosomes; one formed of the fusion of two long arms and the other formed of the fusion of two short arms. Usually the short chromosome formed from their short arms is lost (Fig. 24).

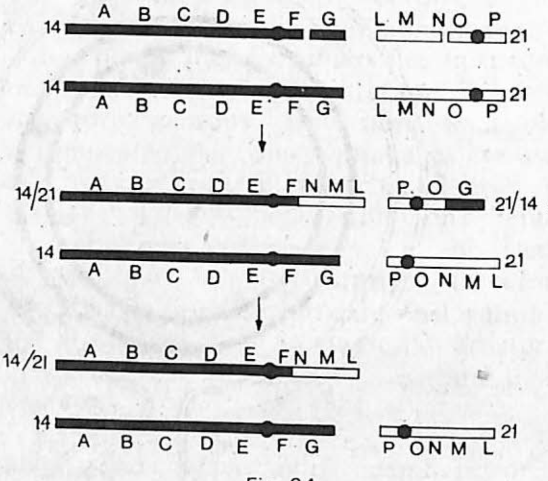

Fig. 24

Formation of Robertsonian translocation or centric fusion. The acrocentric chromosomes 13, 14, 15 and 21, 22 are commonly involved. The chromosome formed by fusion of two long arms (A B C D E F N M L) persists, whereas the chromosome formed by fusion of two short arms (P O G) is lost during subsequent cell divisions, as it is essentially made up of constitutive chromatin which lags behind in duplication.

Robertsonian translocation is so named in honour of the pioneering work of **Robertson,** on this phenomenon in grasshoppers. It is also encountered in many animals. This phenomenon is sometimes referred to as centric fusion, which is a misnomer since it implies, incorrectly, that centromeres fuse. The better term suggested is whole arm transfers.

Robertsonian translocation has served during evolution to produce differences in chromosome number and morphology. In many organisms, the chromosomal area on both sides of the centromere consists largely of the relatively inert heterochromatin; hence the fragment produced by fusion of the short arms consists almost entirely of heterochromatin. The heterochromatin tends to replicate later than the predominant euchromatin. The late replication and the relative inertness of heterochromatin are thought to explain why the short fragment is frequently lost.

The incidence of Robertsonian translocation in man is about 1 per 1,000 births. It usually involves chromosome 14, the majority being interchanges between 13 and 14. About 2 per cent of all cases of Down's syndrome have Robertsonian translocation 14/21, 21/22 or rarely 21/21.

A carrier of Robertsonian translocation is phenotypically normal, because the translocation chromosome contains nearly all the genetic material of the long arms of two acrocentric chromosomes. Only the shorter fragment is lost. The individual is said to carry the translocation in balanced location, as he/she shows no effect of the lost short fragment.

However, during gametogenesis, in a carrier of Robertsonian translocation, imbalance results. During meiosis, as seen in Fig. 25, the translocation chromosome pairs with two normal homologous chromosomes, showing modified trivalent or cross figure, for crossing over. Because of random segregation 2/3 gametes tend to be abnormal, accounting for many stillbirths and abortions. The 1/3 gametes produce viable progeny, but half of them become the carriers of balanced Robertsonian translocation.

Philadelphia Chromosome — Ph^1

Nowell and **Hungerford** discovered that patients with chronic myelogenous or myelocytic or granulocytic leukaemia (CML) have a G chromosome, the long arm of which is shorter than in the usual chromosome. This chromosome was named the Ph^1 (Philadelphia) chromosome after the city of its discovery. This represented the first specific association between chromosomal aberration and cancer.

The chromosome involved is chromosome number 22. Ph^1 is not a simple deletion. The detached piece gets translocated to the tip of the long arm of a number 9 chromosome. Those patients of CML, who lack G deletion, invariably lack the extra material on 9q as well. In about 5 per cent of Ph^1 positive cases, the extra material is on a chromosome other than number 9; and in about 1 per cent of Ph^1 positive cases, no extra material can

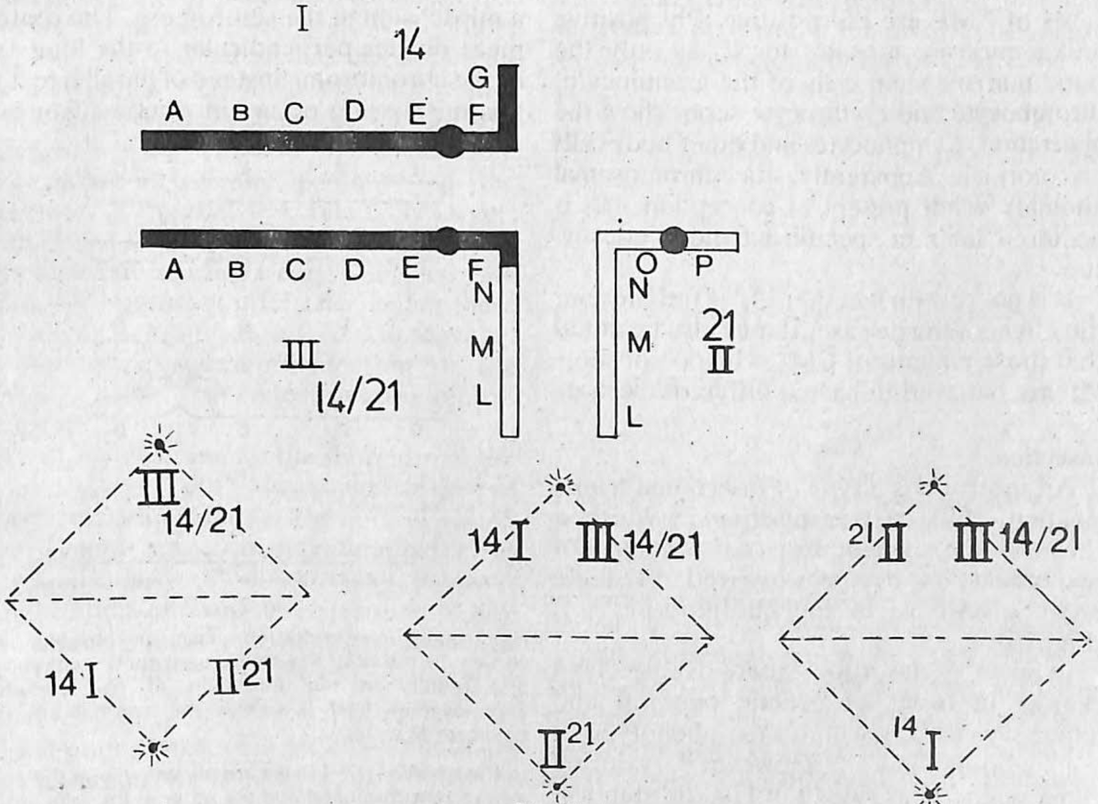

Fig. 25

During gametogenesis, the chromosome with Robertsonian translocation pairs with the two homologous chromosomes whose long arms it represents. Because of random segregation, the resultant four gametes are abnormal (I (14), III (14/21); II (21); II (21), III (14/21); I (14)) one gamete is balanced (III (14/21)) and only one gamete is normal (I (14), II (21).

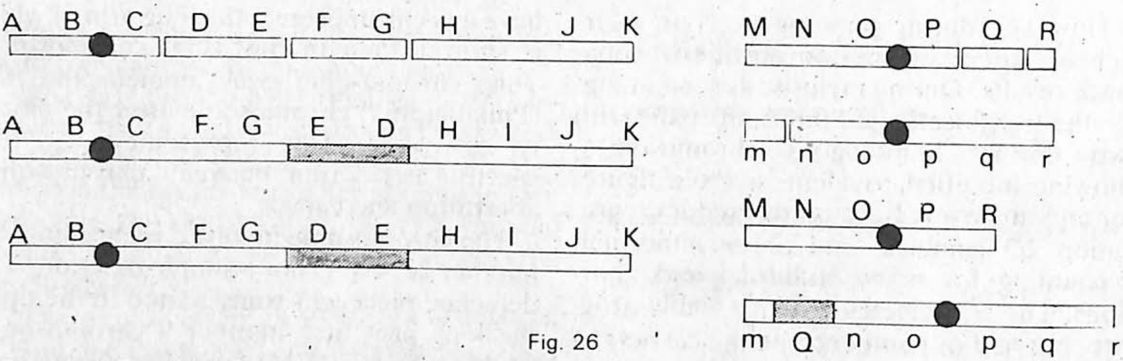

Fig. 26

Insertion. One the left, three breaks are shown. The fragment D E is shown 'inserted' within the same chromosome, after reversal to E D, or as originally broken as D E. This results in a disarrangement of loci.

On the right three breaks are shown, but here, the fragment marked Q gets inserted into the other member of the homologous pair between loci m and n. Again a disarrangement of loci takes place.

be detected elsewhere.

About 85 per cent of those diagnosed as cases of CML are Ph^1 positive. Ph^1 positive leukaemics are mosaics for it, as only the bone marrow stem cells of the granulocyte, thrombocyte and erythrocyte series show the aberration. Lymphocytes and other body cells are normal. Apparently, the chromosomal anomaly is not present at conception, but is acquired later in specific haemopoietic tissue.

It is not certain whether Ph^1 is the cause or the effect of the disease. It may also be noted that those patients of CML who do not show Ph^1 are believed to have a different disease.

Insertion

An insertion is a type of **insertional translocation, shift** or **transposition** in which a chromosome segment drops out as a result of two breaks, but becomes inserted at a single break elsewhere in the chromosome set. (Fig. 26).

In case of insertion, there is merely a change in order of genetic material and hence no effect is seen on the phenotype of the individual. However, during gametogenesis as shown in Fig. 26, duplications or deficiencies result when crossing over involves the insertional segment, or a balanced condition, if crossing over does not cover the insertional segment.

Isochromosomes (Fig. 27)

A new type of chromosome may arise from a misdivision at the centromere. The centromere divides perpendicular to the long axis of the chromosome instead of parallel to it. If this misdivision occurs in a submedian cen-

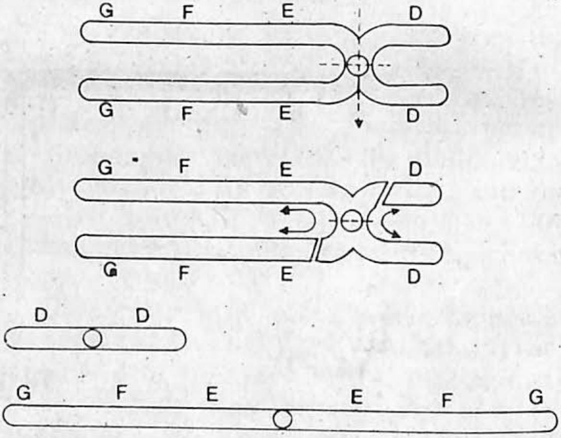

Fig. 27

Isochromosome formation. Two mechanisms are shown. In the first, the centromere splits transversely and results in the formation of two abnormal chromosomes. Each is metacentric, and has an exact duplicate of its loci.

In the second, two breaks are shown, one in the short arm of one chromatid and the other in the long arm of the sister chromatid, both breaks being very close to the centromere. The broken fragments interchange places on the sister chromatids where they get reunited. This again results in the exact duplication of loci on the short and the long arms.

tromere (e.g., that of an X chromosome), the chromosome instead of dividing into identical halves, divides into a long and short chromosome, both with metacentric centromeres; these are isochromosomes — chromosomes with identical arms, partly duplications and partly deletions of the normal chromosome.

Fig. 27 shows that isochromosomes could also result from a whole-arm centric fusion, e.g., in early anaphase, between the strands that were sister chromatids until that stage.

At meiosis the isochromosome pairs with itself or with a normal homologue.

Isochromosome for the long arm of the X chromosome implies absence of all, or most of the short arm(s); and since the short arm appears to contain the genetic material necessary for normal sexual development, the individuals with long arm isochromosomes phenotypically manifest 45, XO like individuals with Turner's syndrome. the only difference is the presence of the chromatin body, that too, relatively larger; there is also a somewhat lower frequency of webbing and cardiovascular defects. An additional significant difference is that these individuals exhibit a tendency to develop chronic lymphocytic inflammation of the thyroid, the so-called Hashimoto's thyroiditis.

An isochromosome for the short arm of the X chromosome, 46Xi(Xp) has a double dose of genetic material essential for normal sexual development; hence the individuals with this isochromosome do not show an external manifestation of Turner's syndrome. They have streak gonads and resemble those, in whom there has been deletion of the long arm of the X chromosome.

Isochromosomes of the autosomes are most numerous for the acrocentric or nearly acrocentric chromosomes. Many are really centric-fusion translocations between homologous chromosomes, as a result of which all the offspring tend to be trisomic or monosomic. The result depends on which chromosome happens to be involved. Most of the autosomes are lethal in both monosomic and trisomic conditions. However, for a chromosome like 21, where trisomy (Down's syndrome) is viable, a high frequency of trisomic siblings are expected in the progeny of a carrier of such an aberration.

Inversion

When there occur two chromosomal breaks on the same chromosome and the segment between the two breaks reforms after rotating through 180°, an inversion is formed. This may take two forms: (1) **paracentric inversion** if the inverted segment does not include the centromere and (2) **pericentric inversion** if the involved segment includes the centromere region (Fig. 28).

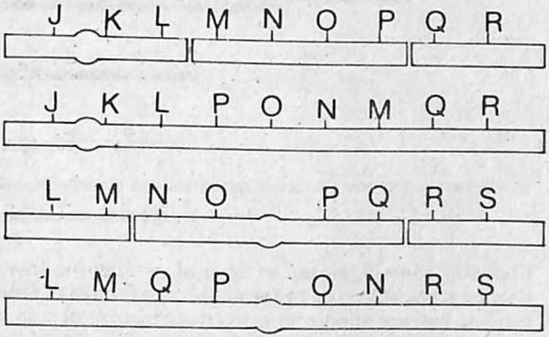

Fig. 28

Inversion. Two breaks are shown. In the first the centromere is not involved. The broken fragment M N O P gets reversed and rejoins the other two fragments. This is known as paracentric inversion.

In the second, the middle fragment which includes the centromere, gets reversed and reattaches to the other two fragments. This is known as pericentric inversion.

The incidence of pericentric inversion is about one per 7500 births, about 1/15 the incidence of translocation of all types. Usually a change in the gene order produced by an inversion does not lead to an abnormal phenotype. However, the effect is seen during gametogenesis. As shown in Figs. 29 and 30, the carriers of inversion produce unbalanced gametes, and are therefore reproductively inferior to the general population,

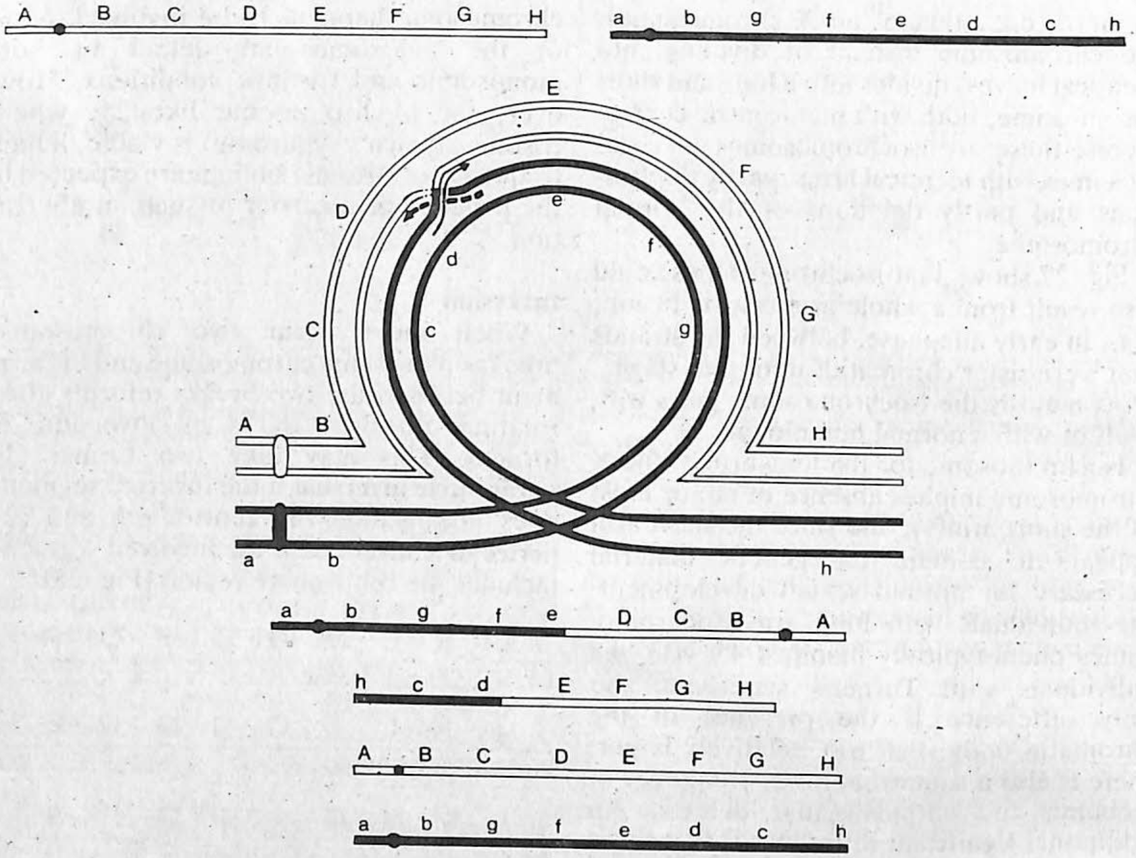

Fig. 29

Showing gametogenesis in case of paracentric inversion. Since some loci are mismatched, crossing over becomes possible only by formation of a loop to enable a rematch of the 'inverted' loci. Of the four gametes, so formed, two are abnormal with an increase or decrease in the number or loci (duplication and deletion), and the other two retain the original number and order of loci.

unless some mechanism exists to reduce crossing over in such cases.

Duplication

It is the presence of a portion of a chromosome more than once in a gamete or more than twice in a zygote.

A. Duplication results from gametogenesis in a carrier of translocation, of inversion or of ring chromosme. Figs. 21,23,29,30.

These duplications do not usually have as drastic an effect as deficiencies of comparable size. Thus, a small duplication is rarely lethal, even when homozygous, whereas small deficiencies may be lethal even in the heterozygote state.

B. **Repeat** or **tandem duplication** arises from **unequal crossing over.** This is the result of an accident, where two homologous chromosomes do not pair exactly before crossing over takes place. As shown in Fig. 31, the result is a small deficiency on one chromosome and a small duplication on its homologous member.

Being small, the duplication is likely to be an evolutionary mechanism for the acquisition of new genes. With the normal genetic material present adjacent to it, the duplicated area is dispensable. It may mutate to produce a new substance without the danger of the cells being deprived of their normal product. Thus duplicated genes evolve into

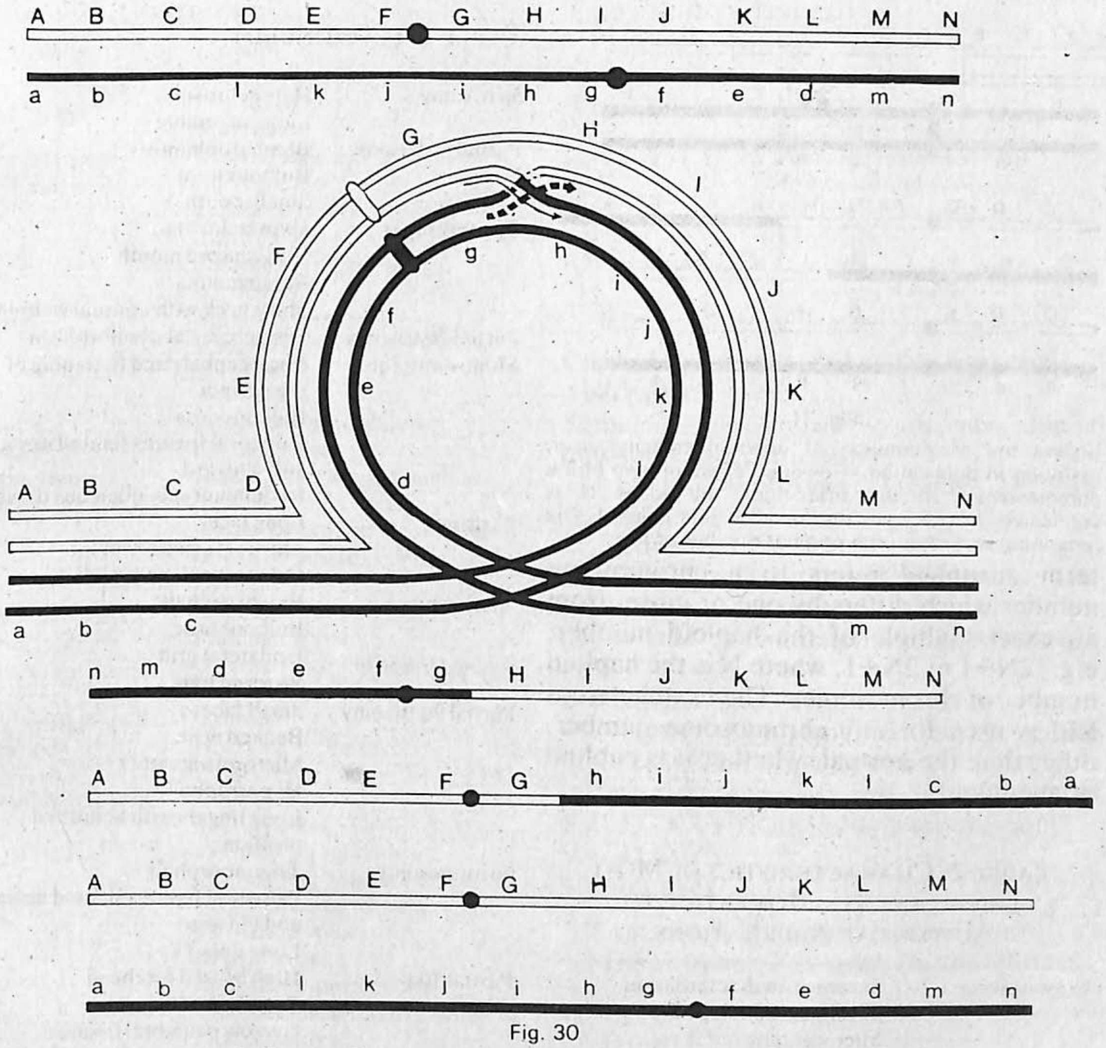
A B C D E F G H I J K L M N
a b c l k j i h g f e d m n
G H I F g h i J f j E e k K A B C D d l L M N
a b c m n
n m d e f g H I J K L M N
A B C D E F G h i j k l c b a
A B C D E F G H I J K L M N
a b c l k j i h g f e d m n

Fig. 30

Showing gametogenesis in case of pericentric inversion, Again, during crossing over, a loop needs to be formed. The resulting gametes with deletion, duplication and retention of original number of loci respectively, are shown.

genes having quite different functions from those from which they originated. Examples are haemoglobin loci, Lepore haemoglobin, and haptoglobins. (Fig. 51).

Abnormalities in Chromosomal Number

The number of chromosomes is fixed for a given species. In man the diploid number in somatic cells is 46 and haploid number is 23. The cause for aberrations in chromosomal numbers in almost all cases is nondisjunction during meiosis I and/or meiosis II, or mitosis.

Euploid is any number which is an exact multiple of the haploid number. The normal species number is **diploid.** However euploid numbers may be abnormal. 3N **(triploid)** and 4N **(tetraploid) chromosome numbers are uncommon in man. Most of these are lethal and hence seen only in early abortuses.** The term **polyploid** refers to any multiple of the basic haploid chromosome number, other than the diploid; thus 3N, 4N and so on. the

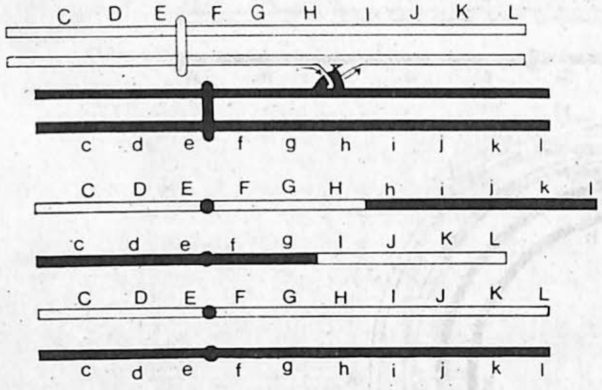

Fig. 31

Shows the phenomenon of unequal crossing over, resulting in duplication — deletion effect on two of the chromosomes. In the first (top), the locus H is duplicated. In the next, the locus H gets deleted. The remaining two retain the original number of loci.

term **aneuploid** refers to a chromosome number which differs by one or more, from an exact multiple of the haploid number, e.g., 2N−1 or 2N+1, where N is the haploid number of chromosomes. The term **heteroploid** is used for any chromosome number, other than the normal, whether it is euploid or aneuploid.

TABLE 2: CHARACTERISTICS OF MOST IMPORTANT SYNDROMES DUE TO AUTOSOMAL ABERRATIONS.

4p monosomy	Severe growth retardation Severe encephalopathy Microcephaly 'Greek warrior helmet'
4p trisomy	Aplasia of the nasal bones 'Boxer nose' in the adult
Partial 4q trisomy	Absence of the nasal bridge 'Cul de poule' or pursed mouth
Partial 4q monosomy	Deformed ears Snub nose
5p monosomy ('Cri du chat' syndrome)	*In the infant* Characteristic cry Microcephaly Moonlike face Hypertelorism *In later years* Narrow face Small mandible, effacement of the angles of the jaw
5p trisomy	Hypotelorism Large mandible
Partial 6p trisomy	Blepharophimosis Bulbous nose Small mouth
Trisomy 6qter	Hypertelorism Bow-shaped mouth Micrognathia Short neck with unusual webbing
Partial 7q trisomy	Discrete facial dysmorphism
Monosomy 7qter	Microcephaly and flattening of the occiput Bulbous nose Palpebral fissures slanted upward and outward Redundant subcutaneous tissue
8 trisomy	Long face Thick, everted lower lip Osteoarticular anomalies
9p trisomy	Brachycephaly Bulbous nose Unilateral grin Worried look
Partial 9q trisomy	Small face Beaked nose Microretrognathia Hypotrophy Long fingers with abnormal position
9p monosomy	Trigonocephaly Palpebral fissures slanted upward and outward Long upper lip
Partial 10q trisomy	High bossed forehead Broad, flat face Narrow palpebral fissures Joint laxity
10p trisomy	Severe growth retardation Dolichocephaly Turtle's beak Osteoarticular anomalies
Partial 11q trisomy	Fleshy nose Protuberant philtrum Microretrognathia Flexed limbs
Partial 11q monosomy	Trigonocephaly Coarse features
Partial 12p monosomy	Growth retardation Microcephaly Narrow forehead Pointed nose Micrognathia

TABLE 2: (CONTINUED)

13 trisomy	Harelip
	Microphthalmia
	Hexadactyly
	Early death
Partial trisomy 13q proximal	Microstomia
	Micrognathia
	Increased nuclear projections of neutrophils
	Abnormal embryonic haemoglobin
distal	Microtrigonocephaly
	Long curly eyelashes – Bushy eyebrows
	Polydactyly
	Congenital heart defect
Distal 13 monosomy (deletion or ring)	Greek profile
	Rabbit-like, forward slanting incisors
	Absent or hypoplastic thumbs
	Bony syndactyly IV-Vth metacarpals
Partial trisomy 14q: proximal	Hypotelorism
	Prominent nose
	Unusual large and mobile mouth
	Thin lips
Proximal 15 trisomy	Oval face
	High cheekbones
	Deep orbits
18 trisomy	Deformed ears
	Micrognathia
	Occipital protuberance
	Overlapping fingers
	High frequency of arches (dermatoglyphic)
	Narrow pelvis
	Rocker-bottom feet
	Considerable growth retardation
	Premature death
18p monosomy or 18p– syndrome	Small size
	Round face
	Broad, detached ears
	Wide mouth
	Dental anomalies
Partial 18q monosomy or 18q– syndrome	Depressed midface
	Carp-shaped mouth
	Strongly folded ears
	High frequency of whorls (dermatoglyphic)
21 trisomy	Hypotonia
	Round flat face
	Palpebral fissures slanted upward and outward
	Brushfield spots on the irises
	Small ears
	Flat nape of the neck
r(21) syndrome	Hypertonia
	Protuberant nasal bridge
	Palpebral fissures slanted downward and outward
	Protuberant occiput
	Large ears
r(22) syndrome	Doe eyes
	Low-set eyebrows

Clinical Syndromes due to Chromosomal Aberrations

Some of the known syndromes due to autosomal aberrations are:

1. Down's syndrome (Trisomy 21, Mongolism)
2. Trisomy 18
3. D trisomy (trisomy 13)
4. *Cri du chat* syndrome.

Those due to anomalous sex chromosomes are:

5. Mosaicism
6. Turner's syndrome (ovarian dysgenesis)
7. 47, XXX (Triple X) and other X polysomies
8. 47, XXY (Klinefelter's syndrome)
9. 47, XYY
10. Intersex.

TABLE 3: A CLASSIFICTION OF VARIOUS DISORDERS OF SEXUAL DEVELOPMENT

1. Seminiferous tubule dysgenesis (Klinefelter's syndrome) XXY, XXXY, and others
2. Ovarian dysgenesis (Turner's syndrome) XO
3. Pseudohermaphrodite
   - (a) male pseudohermaphrodite (XY) testicular feminization syndrome
   - (b) female pseudohermaphrodite (XX) adrenogenital syndrome
4. True hermaphrodite XX, XX/XY, XX/XO and others

Down's syndrome (Trisomy 21, Mongolism) (Fig. 32)

This is the best known of the syndromes with chromosomal anomalies. It was also the first syndrome to be described in humans.

The incidence of 21 trisomy is 1.45 per thousand. This frequency is constant in different ethnic or socio-economic groups. The sex ratio is approximately 3 males to 2 females.

Clinical features. Children with Down's syndrome can be usually identified at birth, or shortly thereafter, by mental retardation: (IQ 25 to 50), brachycephaly, presence of epicanthal folds, oblique palpebral fissure, speckled iris, low bridge of nose, protruding furrowed tongue lacking a central fissure, and small stature. The voice is guttural, low-pitched; articulation is generally defective. Puberty occurs at a normal or slightly delayed age in both sexes. Secondary sexual characteristics are weakly manifested. Females with Down's syndrome can be fertile, although pregnancies are not frequent. Their progeny comprise an equal number of non-trisomic and trisomic children. The non-trisomic children, even with normal karyotype, are mentally deficient. In males with Down's syndrome, no case of fertility has been reported.

Fig. 32
Down's syndrome. Note typical mongoloid facies.

A number of associated anomalies are seen in cases of Down's syndrome. They include congenital heart defects, congenital duodenal obstruction, hypotonia, hyperlaxity of ligaments, dislocation of patella, *pes varus,* strabismus and congenital cataract. The individuals with Down's syndrome have an **increased risk of developing leukaemia.**

Dermatoglyphics. The hands are very characteristic, being short and broad, with single palmar crease **(simian crease)** (Fig. 33) and **clinodactyly (incurving)** of the fifth finger. The hands also have characteristic dermal patterns, including a distal axial triradius. The feet show a wide gap between first and second toes, with a furrow extending backward along the plantar surface. The hallucal area shows characteristic dermal patterns.

Cytogenetics. Down's syndrome involves **trisomy for chromosome 21,** but about 4 per cent of the patients have this extra chromosomal material, not as a separate chromosome, but as a **translocation** of the long arm of chromosome 21 on to either a D (14 or 15) or another G chromosome (21 or 22). A patient with a Dq Gq translocation has 46 chromosomes in all, including the translocation chromosome. This karyotype is effectively trisomic for chromosome 21 and its phenotype is indistinguishable from standard trisomy 21. About 1 per cent of Down's syndrome patients are mosaics, usually with a mixture of 46-chromosome and 47-chromosome cell lines.

The risk, that a woman who has already had a child with Down's syndrome, would have another at a later pregnancy, varies with various factors: the woman's age (higher incidence with **late maternal age**), the karyotype of her child (higher risk with D/G translocation), of her husband **(carrier of**

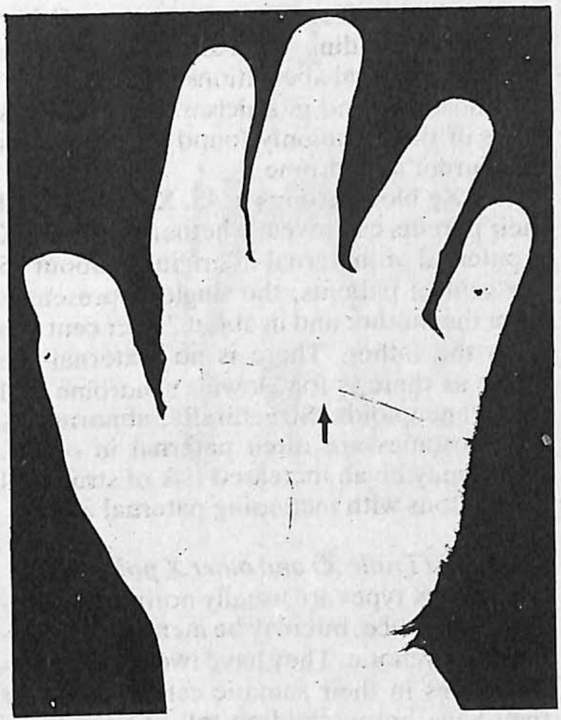

Fig. 33

Down's syndrome. The hand showing single palmar (Simian) crease.

translocation), and of herself (carrier of translocation) and the family history with respect to Down's syndrome.

Trisomy 18 (E syndrome)

This is a syndrome of multiple congenital malformations associated with trisomy for an E-group chromosome, no. 18. It is more severe than Down's syndrome, most of the affected infants dying by the age of six months.

D Trisomy (Trisomy 13)

This is less commonly seen, as anomalies associated with this trisomy are more severe. The commonly associated anomalies are those of the central nervous system, heart, viscera, genitalia and dermal pattern, cleft lip and palate, and polydactyly.

Cri du chat syndrome

This syndrome is due to partial monosomy—deletion of a portion of the short arm of chromosome 5(5p–). It is so called because of a fancied resemblance of the infant's cry to the mewing of a cat. The affected children show mental retardation, microcephaly, characteristic facial appearance with marked hypertelorism, and characteristic dermal patterns in the palm and the sole.

Syndromes due to sex chromosomal anomalies

Mosaicism

Some chromatin-positive males are mosaics, usually 46, XY/47, XXY (Table 4). Some females with Turner's syndrome also show mosaicism.

TABLE 4: HUMAN SEX CHROMOSOMAL ANOMALIES

Male	Female	Gynandric
XXY	XO	
XXXY	XXX	
XXXXY	XXXX	
XYY	XXXXX	
XXYY	XY	
XXXYY	XYY	
XX		
Mosaics		
XY/XXY	XO/XX	XO/XY
XY/XXXY	XO/XXX	XO/XYY
XXXY/XXXXY	XX/XXX	XO/XXY
XY/XXY/?XXXY	XXX/XXXX	XX/XY
XXXX/XXXXY/XXXXXY	XO/XX/XXX	XX/XXY
	XX/XXX/XXXX	XX/XXYY
		XO/XX/XY

Turner's syndrome (ovarian dysgenesis)

These phenotypically female patients have 45 chromosomes with only a single X and are chromatin-negative. Those patients of ovarian dysgenesis who are chromatin-positive are mosaics with 45, X/46, XX/47, XXX cell lines or have one normal and one structurally abnormal X.

Approximately one in every 2500–3500

new-born girls has a chromosomal aberration that leads to **gonadal dysgenesis** or **Turner syndrome.** Embryos with the karyotype 45, X are exceptionally prone to abort. About one fifth of chromosomal abnormalities which lead to first-trimester abortions are accounted for by 45,X.

Clinical features. (Fig. 34.) Patients with Turner's syndrome usually have a short stature with webbing of the neck, low hairline at the nape of the neck, characteristic facial appearance, wide chest with broadly spaced nipples, poorly developed breasts, juvenile external genitalia and female internal sexual organs with ovary represented by a streak of connective tissue. Commonly associated features are oedema of the extremities, coarctation (narrowing) of the aorta and cubitus valgus (reduced carrying angle at the elbow). Axillary and pubic hair are usually present. Primary amenorrhea may be the presenting feature (Table 5).

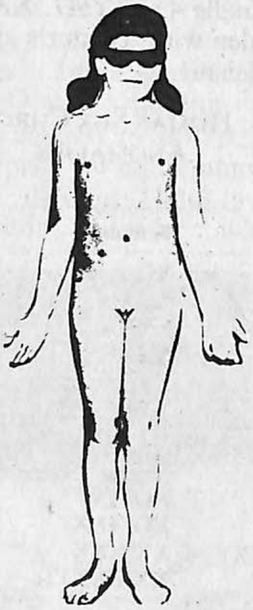
Fig. 34
Turner's syndrome.

Cytogenetics. The karyotype 45,X is responsible for about 40-60 per cent of patients with Turner s syndrome. Other karyotypes leading to Turner's syndrome involve structural aberrations of the X and Y chromosomes and mosaicism. Table 6 gives some of the commonly found karyotypes in the Turner's syndrome.

The Xg blood groups in 45, X patients and their parents can reveal whether the single X is paternal or maternal in origin. In about 75 per cent of patients, the single X present is from the mother and in about 25 per cent it is from the father. There is no maternal-age effect as there is for Down's syndrome and other aneuploids. Structurally, abnormal X chromosomes are often paternal in origin. There may be an increased risk of structural aberrations with increasing paternal age.

47, XXX (Triple X) and other X polysomies. 47, XXX types are usually normal in external appearance, but may be mentally subnormal or psychotic. They have two sex chromatin bodies in their somatic cells. Whenever they have borne children, all of them have been normal. Patients with four or five X chromosomes are also physically normal but show severe mental retardation.

47, XXY (Klinefelter's syndrome) (Fig. 35). The patients are phenotypically males but show presence of sex chromatin.

Approximately one in 1000 new-born boys has a karyotype which is known to be associated with Klinefelter's syndrome.

Clinical features. The patients with this syndrome have small testes showing hyalinization of the seminiferous tubules. Secondary sexual characters are poorly developed and may be associated with gynecomastia. Many patients are tall and eunuchoid. Mental retardation is a commonly associated feature.

It is frequently reported to be associated with diabetes mellitus, thyroid disorders, peptic ulcers, pulmonary emphysema and

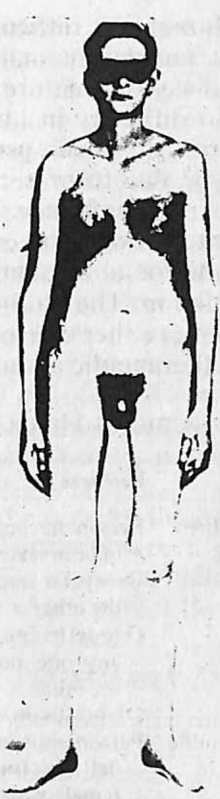

Fig 35

Klinefelter's syndrome.

haemorrhoids. Acute and chronic leukaemias and other cancers are reported more often in association with Klinefelter's syndrome.

Cytogenetics. About 30-90 per cent of all patients with Klinefelter's syndrome have the karyotype 47,XXY. Mosaicism is seen in the remaining 10 per cent, the commonest being 46,XY/47,XXY. The others are 46,XX/47,XXY; 45,X/46,XY/47,XXY; 46,XY/48,XXXY, and 47,XXY/48, XXYY.

Unlike the case of Turner's syndrome, in Klinefelter's syndrome the error is maternal in 64 per cent of cases and paternal in 36 per cent. The error in gametogenesis can occur at either the first or the second meiotic division or at both (seen as rare and more severely abnormal variants of Klinefelter's syndrome with up to four X's).

Table 5: Associated Features of Turner's Syndrome

General
Cubitus valgus
Short metacarpals or metatarsals, usually the IV
Deformities of the medial tibial condyle
Osteoporosis
Head and face
Epicanthal folds
High-arched palate
Abnormal teeth
Visual abnormalities, usually strabismus
Auditory defects, usually due to inner ear defects
Neck
Webbed neck
Short, broad neck with low hairline
Chest
Shield chest with apparently widely spaced nipples
Cardiovascular
Coarctation of the aorta or ventricular septal defect
Renal
Horseshoe kidney
Duplicated or otherwise anomalous ureters
Unilateral renal aplasia or hypoplasia
Gastrointestinal
Telangiectases
Skin and lymphatics
Pigmented naevi
Lymphoedema of hands and feet
Nails
Hypoplasia or malformation

Table 6: Turner's Syndrome and other forms of Gonadal Dysgenesis

Karyotype	**Description**
45,X	XO, Monosomy for X chromosome
46,X,i(Xq)	Isochromosome for the long arm of X; or iso-X
46,X,del(Xp) or 46, XXp-	Deletion of the short arm of X
46,X,del(Xq) or 46,XXq-	Deletion of the long arm of X
46,x,r(X)	Ring X
46,X,i(Xp)	Isochromosome for the short arm of X
46,X,i(Yq)	Isochromosome for the long arm of Y
46,X,t(X;X) or 46,X,ter rea(X;X)	X-X translocation; terminal rearrangement
46,X,t(X;Autosome No.) or 46,X,t(X:Y)	X-autosome or X-Y translocation

47, XYY, Males

Males with this karyotype are indistinguishable from normal males on the basis of their phenotype and behaviour. Some correlation has been found between XYY and aggressive, psychopathic or criminal behaviour.

XYY males originate by paternal non-disjunction at the second meiotic division, which produces YY sperm. XXYY and XXXYY males are less common. Such males also probably originate by paternal non-disjunction by a series of non-disjunctional events.

Intersex

An intersex is defined as an individual with ambiguous genitalia. Intersex conditions are not always due to aberrations of sex chromosomes. They can also be due to single mutant genes. The various types of intersex seen clinically are true hermaphroditism and pseudohermaphroditism. Table 7 gives features of hermaphrodites. The great majority of true hermaphrodites are 46, XX, but some are 46, XX/46, XY chimeras or are 46, XY and have only testicular tissue. Female pseudohermaphrodites are 46, XX and have only ovarian tissue. Male pseudohermaphroditism is due to testicular feminization, which is probably an X-linked disorder and is characterised by unresponsiveness of target organ to testosterone, possibly due to its inability to convert it to its active form, dihydrotestosterone. Phenotypically, the person may have been reared as a male or a female. Female pseudohermaphroditism is commonly due to congenital adrenal hyperplasia. Several distinct genetic and clinical forms of congenital adrenal hyperplasia are seen, inherited as an autosomal recessive. Each disorder is characterised by a block in a specific step in cortisol biosynthesis, resulting in increased secretion of ACTH and hyperplasia of adrenal glands, which leads to masculinization of female foetuses. Affected female children have major anomalies in the genitalia which makes it difficult to identify the child as a female. In males the same genotype produces premature virilization, but there is no difficulty in identifying the sex. Other forms of **female pseudohermaphroditism** maybe due to presence of excessive amounts of male or female sex hormones in the foetal circulation. The excessive hormones reach the foetal circulation from the maternal circulation. The mother has higher levels of hormones either due to overproduction or due to therapeutic administration.

TABLE 7: FEATURES OF HERMAPHRODITES

Type	Features
True hermaphrodite	Person having gonadal tissue of both sexes
(a) Lateral	Ovary on one side, testis on the other
(b) Unilateral	Ovo-testis on one side, and any one normal gonad on the other
(c) Bilateral	Ovo-testis on both sides
Pseudohermaphrodite	Person showing normal gonadal sex (male with testis, female with ovary) and normal sex chromosomal constitution (male with XY, female with XX) but abnormal external and/or internal genitalia tending in varying degree, towards the opposite sex
(a) Female	Person having normal ovary and internal genitalia but external genitalia and secondary sexual characters resembling those of male
(b) Male	Person having functional or non-functional testis with internal and/or external genitalia resembling those of female

Causes of Chromosomal Aberrations

The exact genetic and environmental factors which lead to chromosomal aberrations are not known. What is observed is that some factors predispose to chromosomal aberrations. Late maternal age is a common factor observed in the majority of cases of Down's syndrome but is of less significance in other trisomies. Radiation, viruses, autoimmune diseases and specific genes have been held responsible for non-disjunction or other chromosomal aberrations.

The factors mentioned above are operative for sex chromosomal anomalies also. The basis of all numerical anomalies of sex chromosomes is non-disjunction. The non-disjunctional event occurs at either the first or the second meiotic division in one of the parents during gametogenesis. The non-disjunction occurring in early divisions of the zygote leads to mosaicism.

A study of the gene for the Xg blood group and other X-linked marker genes of the parents and the abnormal child, is useful in finding out in which parent the non-disjunctional event took place.

SUMMARY

Chromosomes may be abnormal either in number or in structure.

Numerical abnormalities are 'euploid' when exact multiples of a chromosome set occur in a cell, or 'aneuploid' when one or more chromosome is more or less than the set.

Structural abnormalities are:

1. Deletion: The elimination of a part of chromosome following a chromosomal break.
2. Ring chromosome: Following terminal breaks, the chromosomal ends join each other.
3. Translocation: This occurs due to breaks in two-nonhomologous chromosomes and the attachment of broken pieces to the wrong chromosome. A mutual 'wrong' exchange is called reciprocal translocation.
4. Isochromosome: A duplication-deletion anomaly due to a transverse split of a metaphase chromosome instead of a longitudinal one. Results in metacentric chromosomes in which their two limbs carry identical gene loci.
5. Inversion: Following two breaks within a chromosome, the segment in between gets reversed and reattached in the same chromosome. The gene loci in the fragment get 'inverted' in sequence. An inversion may include the centromere — 'pericentric' or exclude it – 'paracentric'.
6. Duplication: the representation of a portion or fragment of a chromosome more than twice in a zygote or more than once in a gamete. Usually occurs due to 'unequal crossing over' in meiosis. Can also occur during gametogenesis in a carrier of translocation, of inversion or of ring chromosome.

Numerical abnormalities are:

1. Polyploidy: An euploidy in which an exact multiple other than diploid of a full set of chromosomes of a gamete occurs in a nucleus.
2. Trisomy: An aneuploidy in which one extra chromosome occurs in a nucleus. Examples of trisomy are Down's syndrome (trisomy-21), Klinefelter's syndrome (trisomy XXY).
3. Monosomy: An aneuploidy in which there is one chromosome less than the species specific or 2n number. Example of monosomy is Turner's yndrome (Monosomy X).

Radiation, viruses, some drugs and chemicals, autoimmune disease and late maternal age predispose to chromosomal anomalies. The basis of all numerical sex-chromosome anomalies is non-disjunction, i.e., a failure of separation or detachment from each other, of the paired homologous chromosomes, either at the first or second meiotic division.

QUESTIONS

1. Enumerate the types of structural abnormalities of chromosomes.
2. Explain:
    Deletion
    Duplication
    Translocation
    Inversion
    Isochromosome
    Ring chromosome
3. Enumerate the types of numerical chromosomal abnormalities.
4. Explain:
    Euploidy
    Aneuploidy
    Polyploidy
    Trisomy
    Monosomy
    Polysomy
    Non-disjunction
5. Write notes on the inheritance and characteristic features of:
    Down's syndrome
    Turner's syndrome
    Klinefelter's syndrome
6. Enumerate the known causes of chromosomal aberrations.

4 Molecular Genetics: Genes

INTRODUCTION

Chromosomes contain DNA, the genetic material which is transmitted not only from parent cells to daughter cells during mitosis, but also from one generation to another following meiosis and fusion of male and female gametes. The chromosomes also contain proteins, which constitute the 'non-genetic' but functionally essential material, and small amounts of RNA.

The conceptual unit of heredity is called a gene and, depending upon the aspect from which it is considered, it may be defined in several ways.

Genes are segments of DNA, long or short. They are directly concerned with the formation of all RNAs, including the different tRNA's, the ribosomal subunits, the precursor and the final mRNA and the nucleolar RNA. Through the agency of these RNA molecules, the genes regulate all protein synthesis, structural as well as enzymatic. The proteins, in turn, of course promote or inhibit the activity of the genes.

Genes replicate themselves prior to cell division. They regulate the activity of other genes, which may be in their vicinity or placed more remotely. They can undergo a structural change or mutation and can recombine during the exchange of genetic material in meiosis.

Genes also regulate mitotic activity, cell-growth and cell-differentiation as in embryogenesis, and are suspected to play an important role during the cancerous change of a cell or tissue.

It is evident from what has been said that DNA plays a pivotal role in the **growth, differentiation, metabolism** and **multiplication of cells.**

Small amounts of DNA are also found in the mitochondria and constitute the mitochondrial genes. These are capable of replication and play a role in cellular metabolism.

During embryonic development, it appears that the sequence of events is the result of the **switching on** or **switching off** of particular gene-loci. The precise mechanism of gene regulation is still far from clear. Apparently, some gene products such as proteins function as **messenger molecules** to suppress or activate the genes, as required by the overall economy of the cell. Since all gene-loci are never active at the same time, and the cytoplasmic constituents play a crucial role in regulating gene activity, the cytoplasm is now believed to play a primary role in cell differentiation, which is merely a reflection of differential gene activity.

Chromosomes are composed of deoxyribonucleic acid (DNA) and protein. Variable lengths of DNA constitute the genes, the units of heredity, of which there are about 100,000 in each human cell. The number may vary from 10,000 to several million depending upon the definition of the term gene and the mode of computation.

The genotype of an individual is his genetic constitution. The genome is the full set of genes, haploid in the gametes and diploid in the somatic cells of an individual. The phenotype is the expression of any of these genes as a physical, biochemical or physiological trait.

In this chapter genes will be discussed from various aspects which relate to molecular genetics, and developmental genetics.

Molecular genetics deals with the study of genetic material, deoxyribonucleic acid (DNA), the replication of DNA to produce more DNA, the transcription of DNA into ribonucleic acid (RNA), and the translation of RNA into protein in the form of polypeptide chains. The unidirectional DNA-RNA-protein relationship is called the central dogma of molecular genetics.

Criteria required to be met by genetic material:

1. Genetic material must contain biologically useful information that is maintained in a stable form.
2. Genetic information must be reproduced and transmitted faithfully from cell to cell or from one generation to the next.
3. Genetic material must be able to express itself, so that other biological molecules and ultimately cells and organisms will be produced and maintained. Hence some mechanism must be available for decoding or translating the information contained in the genetic material into its 'productive' form. Thus there would be a molecule, which could not only generate its own kind, but also generate other and new kinds of molecules. In this respect it would differ from 'non-living' matter.
4. Genetic material must be capable of variation. Two sources of change have been recognized in present day genetic systems – mutation and recombination.

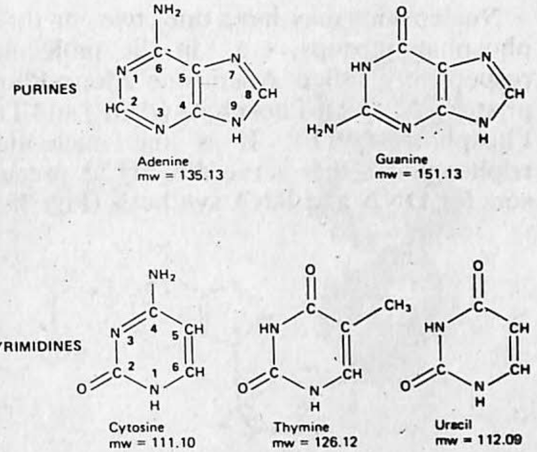

Fig. 36

Purines and pyrimidines, the nitrogen containing bases are the basic components of DNA and RNA.

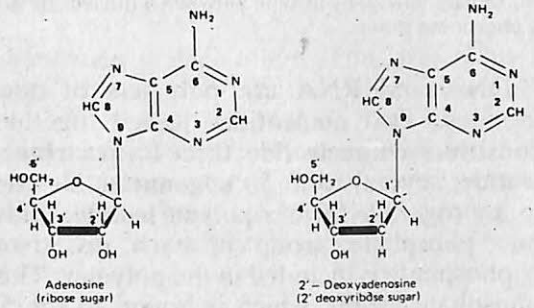

Fig. 37

Two nucleosides formed by linkage between pentose sugar ribose or deoxyribose and purine base, adenine.

Molecular Structure of Nucleic Acids

DNA and RNA are composed of two different classes of nitrogen containing bases, the **purines** and the **pyrimidines.** (Fig. 36). The most common purines in DNA are **adenine** and **guanine** and pyrimidines are **cytosine** and **thymine.** Most species of RNA contain **uracil** in place of thymine (thymine is, in fact, methyluracil). Purines and pyrimidines can form chemical linkages with **pentose (5-carbon) sugars.** (Fig. 37). The carbon atoms on the sugars are designated 1', 2', 3', 4', and 5'. It is the 1' carbon of the pentose that forms the bond to the nitrogen atom in position 1 of a pyrimidine or to the nitrogen atom in position 9 of a purine. The resulting molecules called **nucleosides** can serve as elementary precursors for DNA or RNA synthesis. DNA precursors contain the pentose deoxyribose and the RNA precursors contain ribose instead. Ribose contains an additional oxygen atom at position 2'; this is absent in deoxyribose.

A nucleoside must become complexed with a phosphate group to form a **nucleotide** before it is capable of becoming a part of a DNA or RNA molecule.

Nucleosides may have one, two, or three phosphate groups, e.g., in the molecules respectively called Adenosine Mono-Phosphate (AMP), Di-Phosphate (ADP) and Tri-Phosphate (ATP). It is the nucleotide triphosphates that serve directly as precursors for DNA and RNA synthesis (Fig. 38).

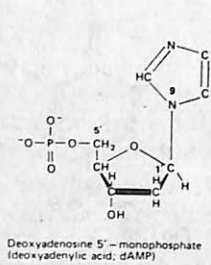

Fig. 38

Nucleotide formed by linkage between a nucleoside and a phosphate group.

DNA and RNA are polymers of nucleotides. Two nucleotides joined together constitute a **dinucleotide**; three form a **trinucleotide**; several form an **oligonucleotide** and many together form a **polynucleotide.** Only one phosphate group of each precursor triphosphate is included in the polymer. This phosphate group, which is bound to the 5' carbon of the pentose sugar on one nucleotide, also gets chemically bound to the 3' carbon of the sugar of a second nucleotide, so that a series of 5' - 3' phosphate linkages, holds the nucleotides together along the length of the polymer (Fig. 39). The phosphate bonds also called phosphodiester bonds are covalent bonds, and so, are extremely strong. Polynucleotides are structurally polarized, meaning that a 3'-hydroxyl (3'-OH) end and a 5'-phosphate (5'-P) end can be identified for any chain. (Fig. 40).

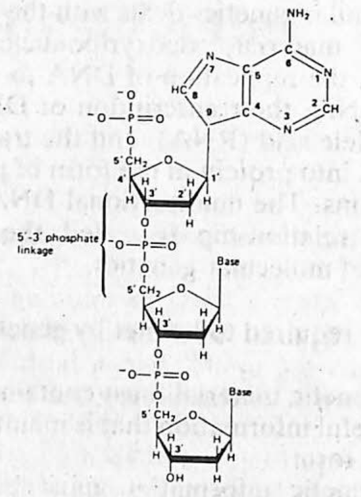

Fig. 39

DNA and RNA are polymers of nucleotides. Here a trinucleotide of DNA is shown, which results from the joining of three nucleotides. Note that the phosphate group, which is bound to the 5'-carbon of the pentose sugar on one nucleotide, also becomes chemically bound to the 3' - carbon of the sugar of a second nucleotide; so that series of 5' — 3' phosphate linkages hold the nucleotides together along the length of the polymer. The phosphate residues along the chain are acidic, whereby the term nucleic acid is also used for the nucleotide chain.

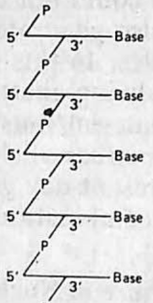

Fig. 40

Structural polarity of DNA because of 5' - 3' phosphate linkages.

As the distance between bases in the polynucleotide chain is 3.4A°, it follows that in the double helix, a strand makes one complete turn every 34A°. The specific hydrogen bonding between base pairs requires that the two polynucleotide strands are oriented in opposite directions. One strand is oriented in 3'-OH→5'-P end and the other is in a 5'-P→3'-OH fashion.

In a cell, the DNA occurs at two sites *viz.*, in the nucleus where it forms the largest bulk, as part of the chromosomes and in the

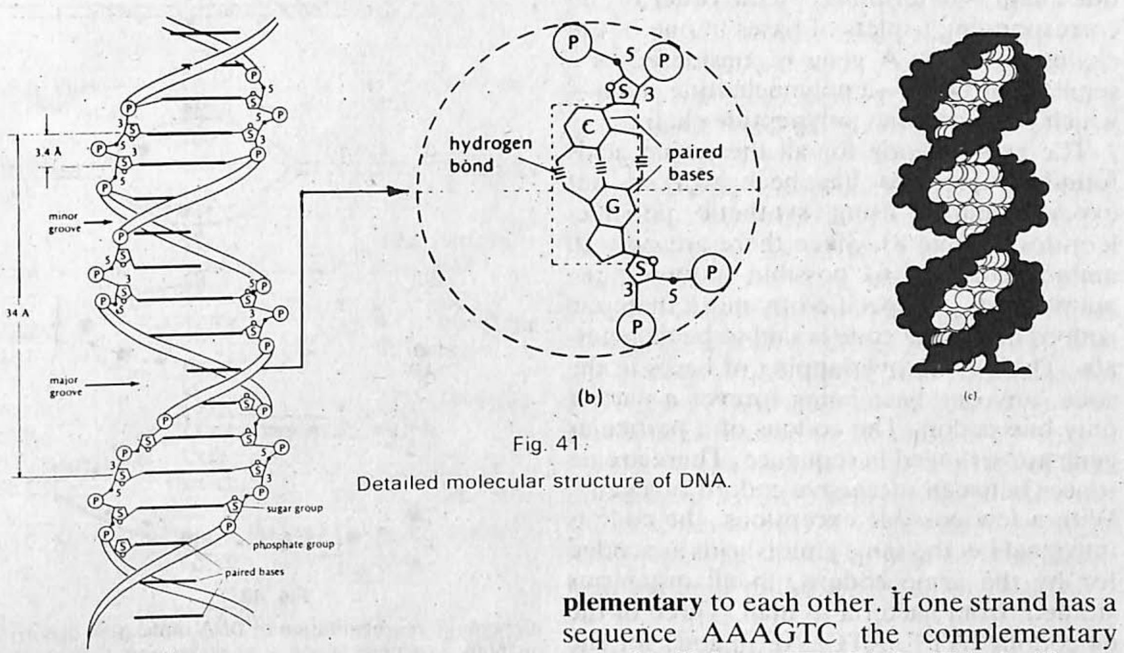

Fig. 41.
Detailed molecular structure of DNA.

cytoplasm, in a very small amount, as part of the mitochondria. Mitochondrial DNA differs from chromosomal DNA in certain details.

In mitochondria, the DNA thread is joined at each end to form a ring (55 S). It has a proportion of nitrogenous bases different from that of chromosomal DNA. Since in a zygote the mitochondria are wholly derived from the ovum, it follows that the few mitochondrial genes in the mitochondrial DNA show strictly **maternal inheritance.**

DNA

DNA molecule is composed of two long, parallel, polynucleotide chains, which are twisted in the form of a **double helix.** (Fig. 41). The chains run in opposite directions and are held together by hydrogen bonds between A of one chain and T of the other, or between G of one chain and C of the other. There are 10 nucleotide pairs per complete turn of the double chain.

As normally A–T and G–C **pairing** is a must, the parallel strands become **complementary** to each other. If one strand has a sequence AAAGTC the complementary strand will have TTTCAG. This facilitates quick and fidel replication of DNA and formation of messenger RNA. Almost infinite variations are possible in the arrangement of the bases along the length of a polynucleotide chain.

The Genetic Code

DNA directs the synthesis of proteins which are molecules made up of amino acids arranged in a specific linear sequence. As there are 20 different amino acids, it is necessary for DNA to provide a code for each one of these. DNA has only 4 nitrogenous bases, which, taken one at a time, could code for only four amino acids. If two sequential bases would code for one amino acid there would be 4×4 or 16 possible combinations which are inadequate for 20 amino acids. If three successive bases would specify one amino acid, then the possible number of combinations would be 4×4×4 or 64. These 64 possible triplet combinations of bases, which constitute a code for amino acids are known as **codons.** The sequence in which amino acids are incorporated into a polypep-

tide chain is determined by the order of the corresponding triplets of bases in one of the chains in DNA. A gene is considered as a segment of DNA—a polynucleotide chain—which codes for one polypeptide chain.

The genetic code for all the amino acids found in proteins has been worked out experimentally, using synthetic polynucleotides. (Table 8). Since there are only 20 amino acids and 64 possible codons, most amino acids are specified by more than one codon; hence the code is said to be degenerate. There is no overlapping of bases in the code, any one base being forever a part of only one codon. The codons of a particular gene are arranged in sequence. There are no spaces between successive codons of a gene. With a few possible exceptions, the code is universal i.e. the same amino acids are coded for by the same codons, in all organisms studied, from bacteria to man. Three of the 64 codons (ATT, ATC, ACT) code for the termination of a gene. The synthesis of a polypeptide chain stops when these codons are read. The codon TAC codes for methionine, and also indicates initiation of a gene. How it functions in this dual capacity is not understood so far.

Replication of DNA

Watson and Crick made an epochal suggestion that the two chains unwind and separate. Each chain serves as a template for the synthesis of its complementary chain. The enzyme DNA polymerase mediates the union of nucleotides during DNA synthesis. Since the two chains are complementary, each synthesises a second chain identical to the one from which it had been separated, and the end result is two complete molecules, each identical to the original. DNA replication occurs in both directions along a chromosome (Fig. 42).

Meselson and Stahl confirmed the Watson-Crick hypothesis of DNA replication, in 1958. They used heavy nitrogen, an isotope of nitrogen. Bacteria were reproduced in a medium in which the only source of nitrogen was N^{15}, so that the DNA of the bacteria became labeled with heavy nitrogen. They were then grown in a medium containing ordinary N^{14} and allowed to reproduce for two more generations. The proportion of N^{15} and N^{14} in the bacterial DNA was measured after each generation. The results are shown in Fig. 43. As the old DNA strands are preserved, and each old strand induces the formation of a new complementary strand, this is referred to as semiconservative replication.

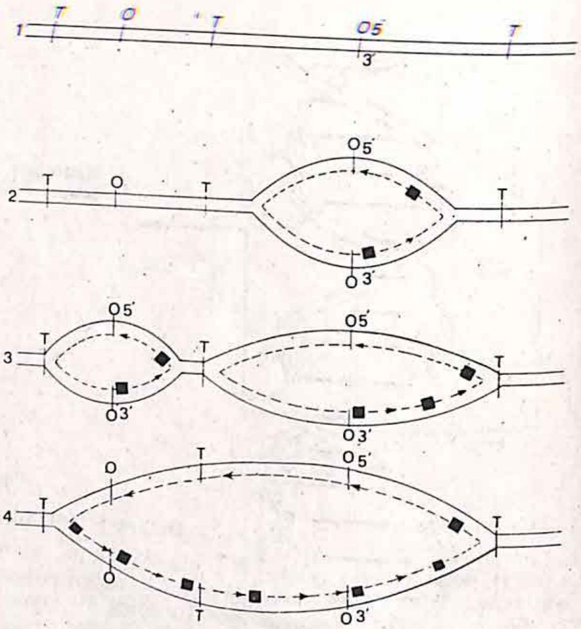

Fig. 42

Schematic representation of DNA replication occurring in both directions along a chromosome. O = point of origin of DNA replication. T = point of termination of DNA replication.

Organization of DNA in Chromosomes (Figs. 7,8,9)

The exact arrangement of DNA in the chromosomes is not known so far. The latest theory speculates that much of the DNA in chromosomes is redundant, yet it is packed in such a manner that it is readily available. DNA combines with a protein—histone. In a

TABLE 8: THE GENETIC CODE

AAA Phenyl-	GGA	TTA Asparagine
AAG alanine	GGG Proline	TTG
	GGT	
AAT Leucine	GGC	TTC Lysine
AAC		
	TGA	CTA Aspartic
GAA	TGG Threonine	CTG Acid
GAG Leucine	TGT	
GAT	TGC	CTT Glutamic
GAC		CTC Acid
	CGA	ACA Cysteine
TAA	CGG Alanine	ACG
TAG Isoleucine	CGT	
TAT	CGC	ACT Stop
TAC Methionine	ATA Tyrosine	ACC Trypto-
	ATG	phan
CAA	ATT Stop	GCA
CAG Valine	ATC	GCG Arginine
CAT		GCT
CAC		GCC
	GTA histidine	
	GTG	
AGA		TCA Serine
AGG Serine	GTT Glutamine	TCG
AGT	GTC	TCT Arginine
AGC		TCC
		CCA
		CCG Glycine
		CCT
		CCC

chromosome, DNA and histone combine in equal proportions.

A human diploid cell nucleus contains about 6×10^9 µm, **base pairs** of DNA. Since 3000 base pairs of DNA occupy a length of 1 micron, the total length of DNA per nucleus is 2×10^6 µm, i.e., 2 metres, whereas the nucleus itself is not more than 10 µm in diameter.

The **human genome** contains about 2.7×10^9 **nucleotide pairs**, which can code for about three million genes. But only a small *percentage of the total genome consists of genes that are transcribed into RNA and* translated into protein, *i.e.,* **structural genes.** It has been estimated that man has about 30,000 structural genes. The function of most of the remainder of DNA is unknown.

The **secondary constrictions (stalks)** of the five pairs of acrocentric chromosomes carry the genes for synthesis of 18S and 28S ribosomal RNA. The stalks of the acrocentric chromosomes are the nucleolus organizer regions of the genome. Numerical aberrations of the acrocentric chromosomes are frequently the causes of abnormality in live newborns or in first-trimester abortions, yet the mechanism by which the risk of nondisjunction is increased in these chromosomes is not known. The genes for synthesis of 5S ribosomal RNA appear to be situated near the ends of the longer chromosomes.

Classification of DNA

Two classes of DNA have been recognised:

1. **Unique** sequence—**nonrepetitive**—DNA constituting 60 to 70% of the genome

TABLE 9: LEVELS OF ORGANIZATION OF THE GENETIC MATERIAL

Component	**Thickness**	**Length**
DNA	2nm or 20A°	2 metres per cell
Nucleosome	8×11nm	—
Chromatin Fibre	25 nm	8 cm. per cell
Chromosome Band or Chromomere	0.4 - 0.8 µm	—
Metaphase Chromosome	0.8 - 1.2 µm	200 - 300 µm per cell or 2 - 12 µm per chromosome

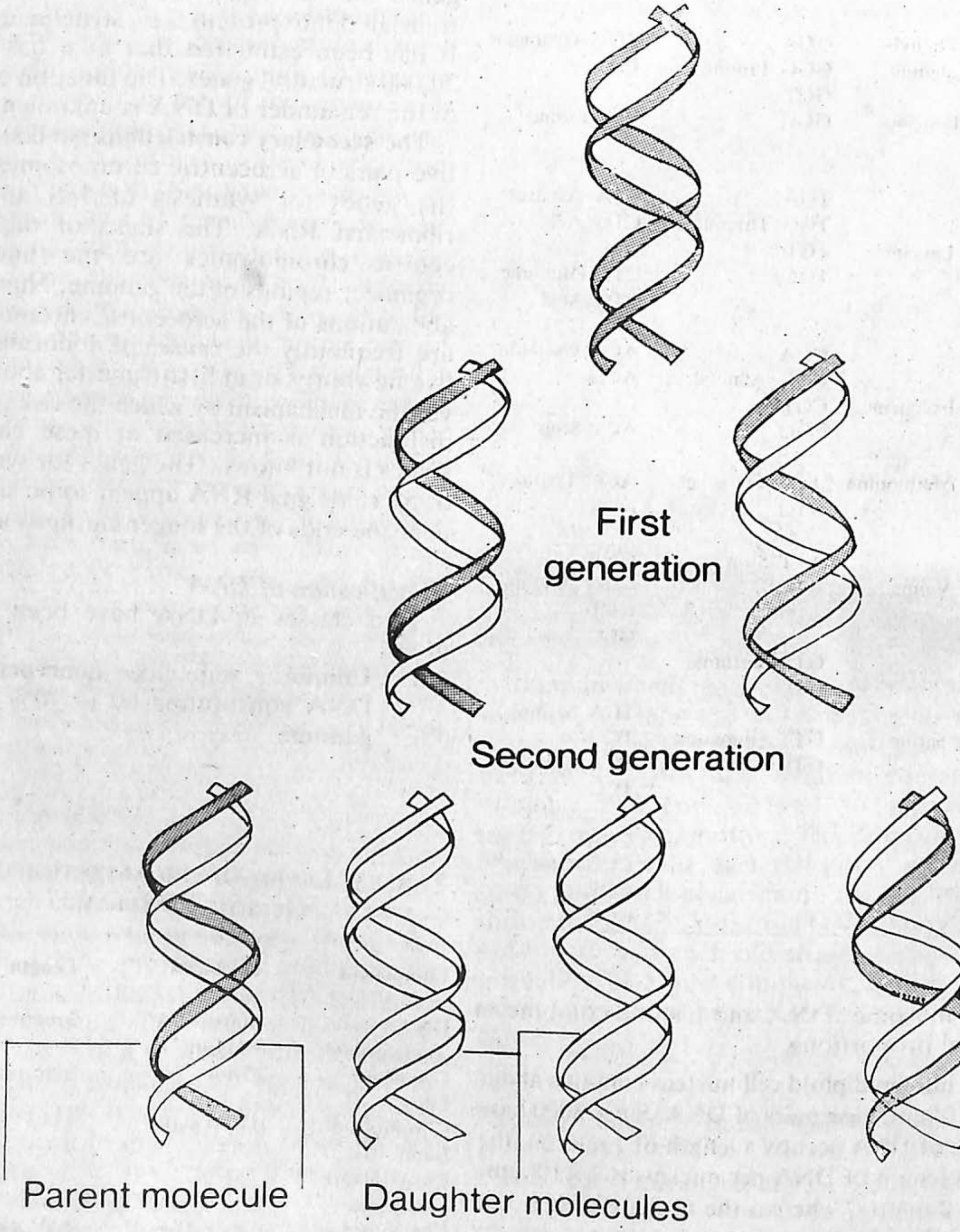

Fig. 43
Scheme showing semiconservative replication of DNA. New complementary strands shown in outlines; old strands shaded dark.

2. **Repetitive** DNA constituting the remaining 30 to 40% of the genome.

Unique sequence DNA accounts for the structural genes. It is also responsible for transcription into heterogeneous RNA and into precursors of transfer RNA and ribosomal RNA. About a quarter of the repetitive DNA is classed as **higher repetitive.** The remaining 3 quarters of the repetitive DNA is interspersed with unique sequence DNA. **Moderately repetitive** DNA sequences are found chiefly in Q and G bands and it is likely that most of the aberrations of large chromosomes in man involve chromosomes with large amounts of such sequences.

Several kinds of a minor DNA component made up of very short, simple sequences which are highly repetitive – occurring in hundreds of thousands or even millions of copies – are referred to as Satellite DNA.

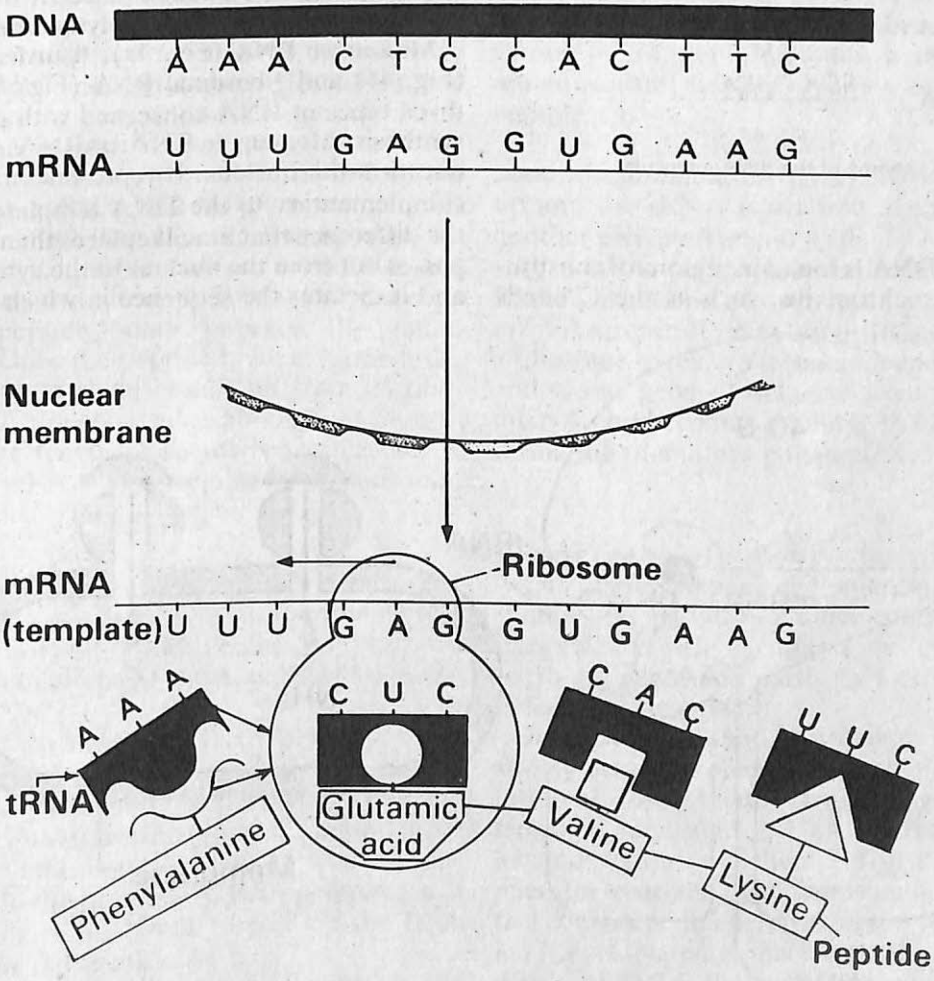

Fig. 44

Transcription and translation. An mRNA molecule is being transcribed along a DNA master strand which acts as a template. In the cytoplasm, the mRNA molecule is being translated by means of ribosomes into specific amino acid linkages. The intermediaries for this are the tRNA molecules, which are specific for each amino acid.

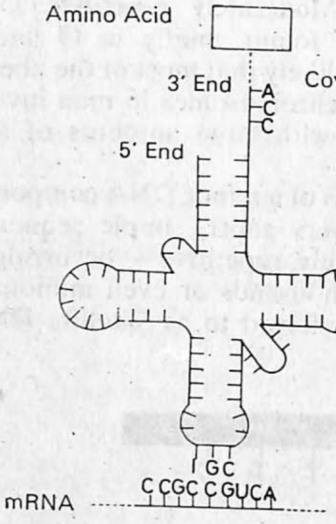

Fig. 45
Scheme of the tRNA molecule.

Satellite DNA is found in regions of constitutive heterochromatin, such as the C bands adjacent to the centromeres or in areas related to the synthesis of nucleolus. It is not transcribed, and is thought to have a regulatory function. Satellite-rich heterochromatin tends to interfere with crossing over and thus helps to preserve the original sequences in the region of the centromere, during meiosis.

RNA

RNA—ribonucleic acid—is composed of a single polynucleotide chain. It is synthesized on a DNA template by a process known as **transcription** which takes place in the presence of the enzyme **RNA polymerase.**

Messenger RNA (Fig. 44), **transfer RNA** (Fig. 45) and **ribosomal RNA** (Fig. 46) are three types of RNA concerned with protein synthesis. Messenger RNA (mRNA) is produced in the nucleus. It represents a strand complementary to the DNA template, with the difference that uracil replaces thymine. It passes out from the nucleus to the cytoplasm and it dictates the sequence in which amino

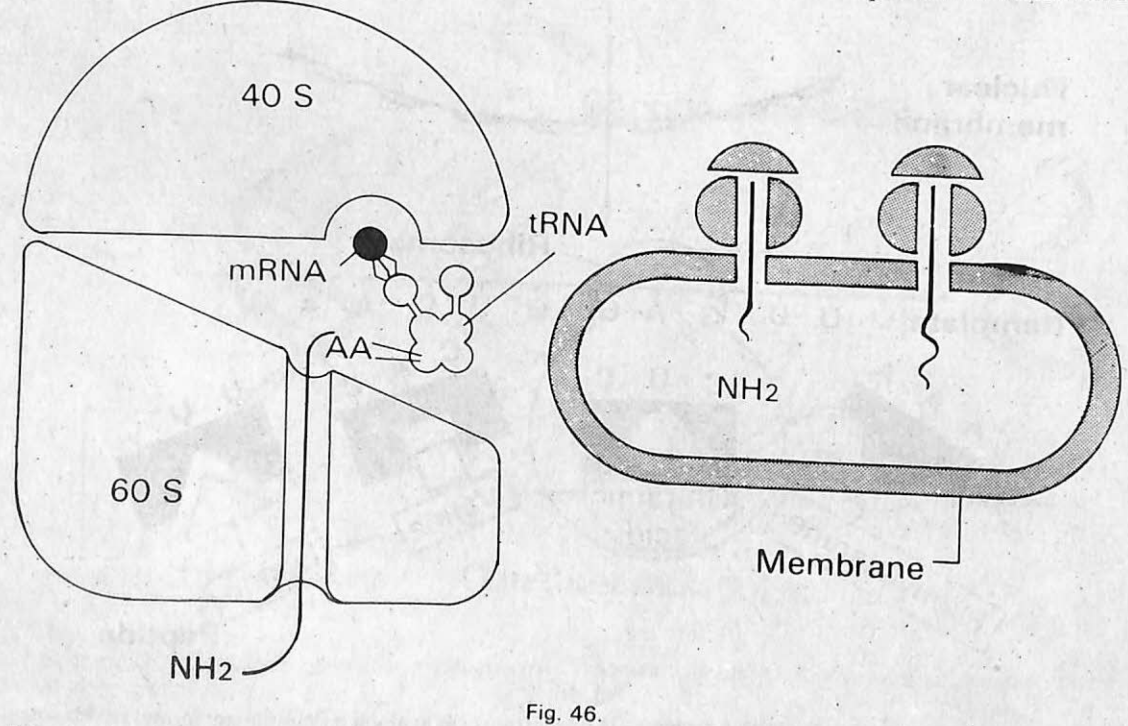

Fig. 46.
Scheme showing the subunits of ribosomes and their relation with protein synthesis. The figure on the right shows a growing polypeptide chain collecting inside the endoplasmic reticulum.

acids are incorporated into a polypeptide. Transfer RNA (tRNA), [also known as soluble RNA (sRNA) because it remains in suspension following centrifugation or as adaptor RNA because of its structure], is concerned with bringing amino acids from the cytoplasm to their required places along the mRNA template. Each amino acid needs an amino acid-activating enzyme, whereby special sites on the amino acid molecule and on the tRNA molecule are recognised. The tRNA molecule has an **anticodon** which is complementary to and reads a specific codon on the mRNA chain. The amino acid-tRNA complex is placed in the correct position on the linear mRNA molecule by the matching of codon and anticodon. **Ribosomes** are made up of protein and non-specific RNA (rRNA) in about equal proportions. The ribosomes are concerned with **reading the code** on mRNA and bringing amino acid-tRNA units into line at the appropriate codons. Ribosomes adhere to mRNA and then proceed along it. Ribosomal enzymes form peptide bonds between the amino acids. Once the peptide bond is formed, the polypeptide chain breaks off from its ribosome. A ribosome takes about 10 seconds to read the length of an mRNA molecule. A single mRNA can have several ribosomes along it at a time.

Protein Synthesis (Fig. 44)

Protein synthesis involves the joining of a number of polypeptide chains. Synthesis of a polypeptide chain involves the following steps:

1. Two strands of DNA helix open out when transcription is needed. No specific enzyme is known to facilitate this. Only one of the two DNA strands acts as a template for RNA synthesis.
2. In the presence of **RNA polymerase,** a molecule of mRNA is formed on the **DNA template** in the nucleus.
3. The mRNA molecule reaches the cytoplasm.
4. In the cytoplasm, amino acids are activated and brought in association with tRNA to form amino acid-tRNA complexes by specific enzymes. Each amino acid has a specific enzyme for activation and for attaching itself to a corresponding tRNA. The tRNA has an anticodon complementary to the appropriate codon of the mRNA.
5. Ribosomes adhere to sticky points on the mRNA molecule and move along the molecule. Under the influence of ribosomes, amino acid-tRNA complexes are brought into a desired order along the mRNA chain, commencing at an AUG mRNA codon.
6. The ribosomal enzyme brings about formation of peptide bonds between the amino acids of successive amino acid-tRNA complexes.
7. With the formation of the peptide bond, the amino acid-tRNA complex breaks up and the tRNA is set free to unite with another activated amino acid.
8. As the polypeptide molecule is being formed, it goes on getting separated from the mRNA strand. It takes about 10 seconds for a ribosome to reach a **terminator codon** at the end of one gene. It detaches itself from the mRNA and becomes available to induce the formation of another polypeptide.

Genetic control of protein synthesis (Fig. 47):

Our present knowledge regarding protein synthesis is based on studies conducted on bacteria. **Protein synthesis** is controlled partly by genes and partly by factors in the external environment.

Jacob and Monod postulated the now widely accepted **operon concept** in 1961. A group of closely related **structural genes (cistrons)** is activated by an **operator gene** located close to them. Together, the **operator gene** and the cistrons under its control constitute on **operon.** Operon is controlled (i.e. activated or inactivated) by two segments of DNA that act as regulatory elements, namely, the **regulatory gene, and the**

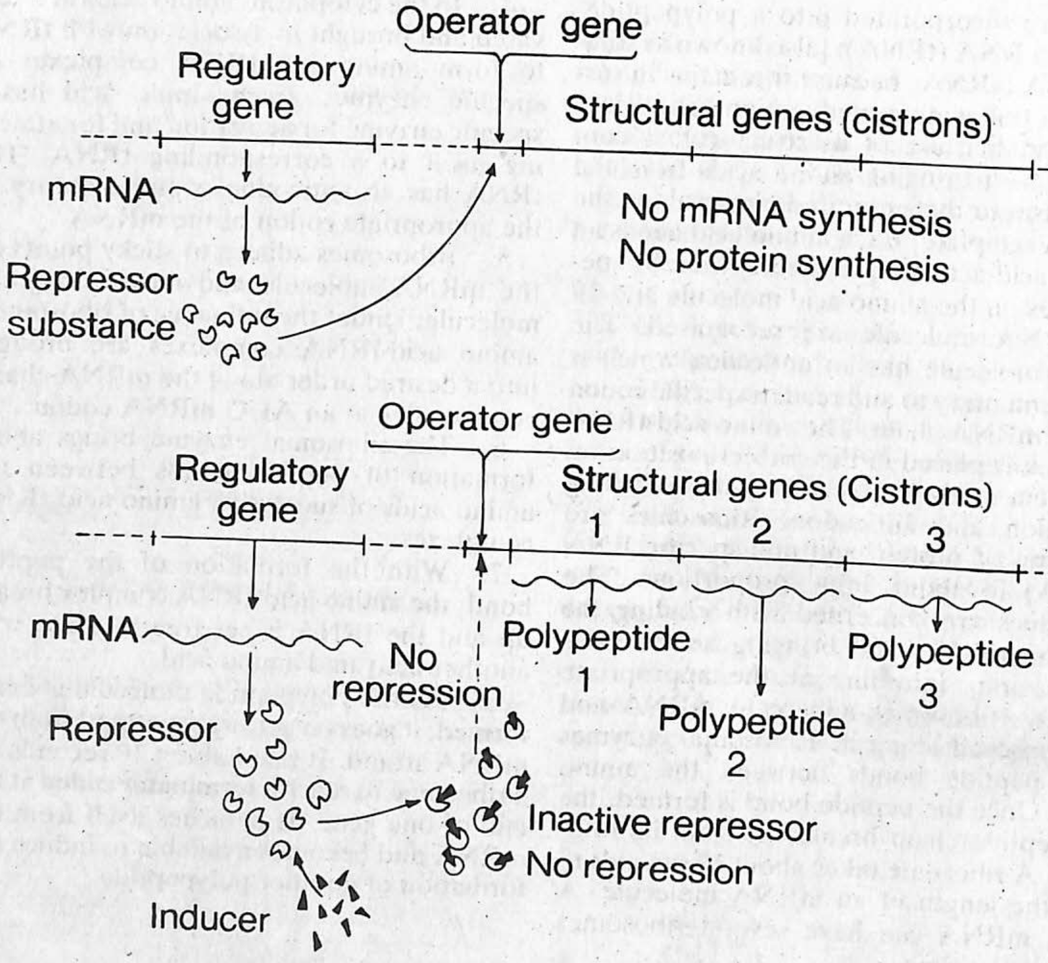

Fig. 47
The Jacob and Monod concept of regulation of protein synthesis.

promoter gene, which may or may not be in the vicinity of the operon.

Normally, only a few genes of the total genome are active. A regulator gene is a DNA segment independent of an operon. It synthesizes a **repressor protein**—a first order messenger—by means of a complementary mRNA strand. Specific second order messengers—**effectors**—presumably similar to the first order messengers, determine the state of activity of the repressor protein. Only when the repressor protein is active can it combine with, and inactivate the operator gene. Some repressors are active and able to block the operator gene only when assisted by **corepressor molecules.** Some second order messengers combine with the repressor so that it is unable to combine with and repress the operator gene thus allowing the related structural genes to become active.

The promoter gene is the DNA segment to which the RNA polymerase first becomes attached to initiate the transcription of the structural genes. The promoter is a key regulatory element, since it controls the rate of mRNA synthesis of a given operon. Both the structural genes and regulator genes have their own promoters.

Only one known operon called the *lac* operon has been studied in *E. coli.* In the *lac* operon, there are three structural genes one each for the enzymes ß-galactosidase, permease, and transacetylase, that are regulated by the same operator; and all of them are involved in the utilization of lactose. The RNA polymerase initiates transcription of the structural genes by binding with the promoter, which is located to the left side of the operator, which in turn is located to the left of the structural genes of the *lac* operon. Therefore, the RNA polymerase is required to negotiate the operator before transcription can occur. Regulation of the *lac* operon is by way of repressor which binds to the operator, thereby interfering with the transcription by RNA polymerase. In this condition, the *lac* operon remains inactive and no enzymes are produced. In the presence of an **inducer** (suitable metabolite in case of *lac* operon), the repressor is inactivated and the operator becomes free. Under such circumstances, the entire operon can be transcribed by the RNA polymerase. The *lac* repressor is a protein of 160,000 daltons with four sub-units of 40,000 daltons, each of which has a binding site for the inducer.

The ***lac* operon** has a positive control mechanism operated by cyclic AMP, which interacts with a receptor protein. The cyclic AMP-receptor-protein-complex gets bound to the promoter, allowing the RNA polymerase to recognise the promoter. When cells are grown in glucose, cyclic AMP levels go down and by a phenomenon known as catabolic repression, ß-galactosidase levels are also brought down.

Gene Structure in Detail

The earlier ideas of the **eukaryotic gene structure** stand considerably revised following the use of the techniques of **restriction endonuclease analysis, recombinant DNA cloning** and **DNA sequencing.** Perhaps the most unexpected discovery has been that the eukaryotic genes exist in pieces, such that the **DNA sequences** coding for a particular protein, the **exons,** are interrupted by one or more sequences of DNA, the **introns,** which do not code for protein. All genes do not contain the same number of introns; and introns as well as exons can vary greatly in length. Each intron or intervening DNA sequence is different; but all introns studied so far show the same base sequences, that is, GT at their 5' end, and AG at their 3' end respectively.

The other similarities in the DNA sequence of eukaryotic genes are: (a) the presence of the sequence TATA, 30 or more nucleotides from the 5' **(upstream)** of the first exon and (b) the sequence AATAAA, several hundred nucleotides from the 3' end **(downstream)** of the last exon. These structural features have an important bearing on the process of **transcription,** i.e., the formation of mRNA transcripts.

Transcription

The sequence TATA, present at the 5' end of eukaryotic genes, appears to be essential for the initiation of transcription (Fig. 48). Sometimes known as the **Hogness box**, it represents a **promoter site** for RNA polymerase II. Mutations or deletions in this sequence reduce remarkably the initiation of transcription of the gene. However, other regions of DNA, often hundreds of bases to the 5' side of the TATA box, can also affect the initiation of transcription. It is suggested that these distinct regulation sequences may influence the spacing or phasing of nucleosome structure in relation to the DNA sequence, which might in turn then determine or not a gene is transcribed.

Capping and polyadenylation

The actual site of initiation of transcription is located about 30 bases to the 3' side (downstream) of the TATA box, at a point which is constant for any particular gene, and is proximal to the 5' end of the first exon. Thus the RNA transcript begins with a 5' region which is not involved in coding for protein. Shortly after the start of transcription, the terminal 5’ nucleotide of the RNA transcript is modified by the addition of a 2’-0 methyl group and a 5’5’ triphosphate-linked 7-methylguanosine residue (m^7GpppX^{m-}). This modified base at the 5’ end of each RNA transcript is known as the **RNA cap.** Following initiation of transcription, the entire length of the gene including both introns and exons gets transcribed. Normally the transcription process proceeds several hundred nucleotides beyond the end of the final or last exon before termination occurs. Thereby, a 3’ non-coding region of the RNA transcript gets created. Following the termination of transcription, a ‘tail’ of polyadenylic acid (poly A) about 100 bases in length, is added to the 3’ end of each RNA transcript. The DNA sequence AAT AAA is almost always found 15 to 30 bases upstream from the site of poly A addition. The precise role played by this AAT AAA sequence in the termination of transcription and the addition of poly A ‘tail’ is yet to be determined. Thus, the primary RNA transcript comes to possess a capped 5’ end, a 3’ poly A tail, and includes both exons and introns, as also 5’ and 3’ non-coding regions.

mRNA splicing

If the primary RNA transcript is to be converted into a functional messenger, RNA (mRNA), it is essential that the **intron-sequences** must be eliminated and the **exon-sequences** should be precisely joined or spliced together (Figs. 48, 49). The **splicing mechanism** has been conserved during the course of evolution. This is suggested by the fact that any eukaryotic cell is able to splice correctly the primary RNA transcripts derived from other species. Also, it has been shown that intron splicing is crucial for the nuclear transport and production of a stable mRNA. The dinucleotides, GT and AG at the 5’ and 3’ ends of the introns, respectively, are critical for proper splicing.

A small RNA molecule called U1 RNA, is found in the form of **ribonucleoprotein particles** in the nucleus. It is remarkable in that its base sequence is highly conserved in different species. It may play a role in **RNA splicing.** Since the ends of the introns are complementary to U1 RNA, it is believed that they may be brought together by base pairing with this RNA, thus looping out the intron and creating a duplex structure which might serve as a substrate for a splicing enzyme. (Fig. 48). These conjectures are supported by the observation that antibodies against U1 RNA-nucleoprotein complexes dramatically inhibit RNA splicing. However, as yet, no splicing enzyme has been recognized.

Translation

The **mature messenger RNA** (mRNA), following the splicing out of the intron sequences from the **primary RNA transcript,** is transported from the nucleus to the cytoplasm where it gets translated into a **polypeptide chain.** In the cytoplasm, the various transfer RNA molecules (tRNA) provide an interface between mRNA and the free amino acids. One site on each tRNA molecule contains a specific sequence of three bases (the anticodon) which permits that tRNA to base pair with the complementary sequence of three bases in the mRNA (the mRNA codon). Another site on the tRNA molecule is recognised by one of the twenty different enzymes (aminoacyl synthetases) which specifically attaches one of twenty amino acids to the tRNA. Thus each tRNA molecule serves as an **adaptor.** It links a specific amino acid to a specific codon in the mRNA. The relationship between the DNA codons and amino acids is determined by the genetic code, as given in Table 8.

All amino acids except methionine and tryptophan, are represented by more than one codon. Some of these codons are used more frequently and preferentially than others for specifying a given amino acid. Since a special tRNA which recognizes the

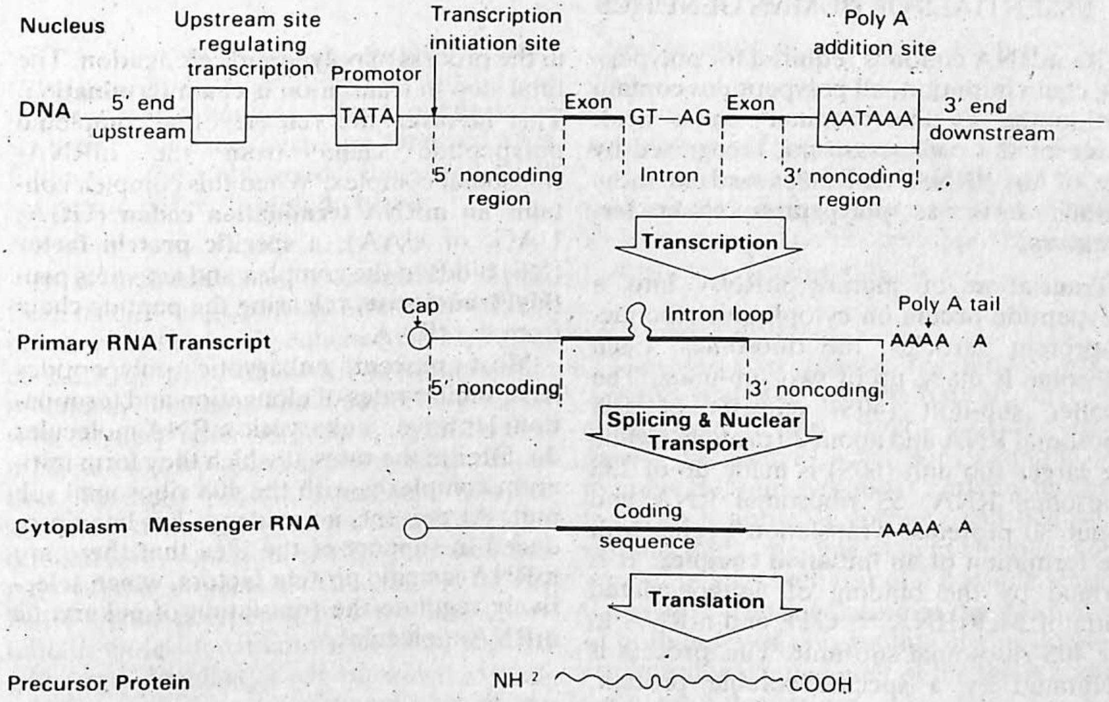

Fig. 48
Eukaryotic gene structure and the steps involved in eukaryotic gene transcription:

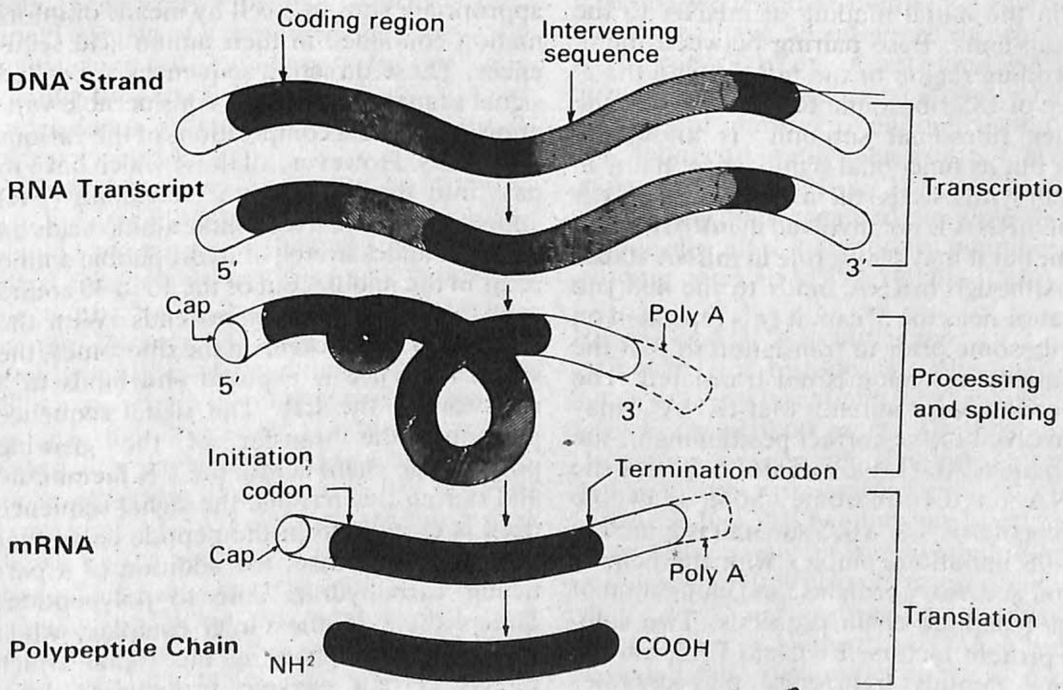

Fig. 49
Schematic summary of genetic expression in transcription — protein synthesis.

AUG mRNA codon is required for polypeptide chain initiation, all polypeptides contain methionine as the terminal amino acid. Three of the codons are not recognised by any of the tRNA molecules and so these codons serve as **polypeptide chain terminators.**

Translation of mature mRNA into a polypeptide occurs on cytoplasmic ribonucleoprotein particles, the ribosomes. Each ribosome is made up of two sub-units. The smaller sub-unit (40S) consists of 18S ribosomal RNA and about 20 proteins; while the larger sub unit (60S) is made up of 28S ribosomal RNA, 5S ribosomal RNA and about 30 proteins. Translation begins with the formation of an **initiation complex.** It is formed by the binding of aminoacylated initiator Met-tRNA^{met}, GTP and mRNA to the 40S ribosomal sub unit. This process is facilitated by a specific soluble protein, designated eIf-2, and a second larger protein, designated eIf-3. The 5' cap structure of eukaryotic mRNA also plays a significant role in the initial binding of mRNA to the 40S sub-units. Base pairing between the 5' non-coding region of the mRNA and the 3' region of 18S ribosomal RNA present in the smaller ribosomal sub-unit, is known to occur but its functional significance, if any in protein synthesis, is still in doubt. The poly A tail of mRNA is not involved in **mRNA translation;** but it may play a role in **mRNA stability.** Although mRNA binds to the 40S sub unit at or near the 5' cap, it gets reposited on the ribosome prior to translation so that the 5' non-coding region is not translated. The anti-codon of the initiator Met-tRNA^{met} may be involved in the correct positioning of the 5' proximal AUG, the initiator codon of the mRNA on the ribosome. Soon after the binding of mRNA, a 60S sub unit is joined to the 40S initiation complex with the help of several accessory proteins, and elongation of the polypeptide chain proceeds. Two soluable protein factors, Ef-1 and Ef-2, and an enzyme, peptidyl transferase; play key roles in the process of polypeptide elongation. The final step in translation is **chain termination.** This involves the release of a completed polypeptide chain from the mRNA-ribosomal complex. When this complex contains an **mRNA termination codon** (UGA, UAG, or UAA), a **specific protein factor** (RF) binds to the complex and activates **peptidyl transferase,** releasing the peptide chain from the tRNA.

Most nascent eukaryotic polypeptides have similar rates of elongation and termination. However, eukaryotic mRNA molecules do differ in the rates at which they form initiation complexes with the 40S ribosomal sub unit. At present, no evidence has been produced in support of the idea that there are mRNA-specific protein factors, which selectively regulate the translation of eukaryotic mRNA molecules.

Protein structure

Signal sequences. Many proteins synthesized on ribosomes are directed to their appropriate sites in a cell by means of information contained in their amino acid sequences. These directing sequences are called signal sequences. There is considerable variation in the exact compositions of the various sequences. However, all those which have to pass into the endoplasmic reticulum (ER) appear to contain hydrophilic amino acids on the ends and a stretch of hydrophobic amino acids in the middle, out of the 15 to 30 amino acid residues at the amino ends. With the elongation of a protein on the ribosomes, the signal sequence is exposed and binds to a receptor on the ER. The signal sequence promotes the transfer of the growing polypeptide chain across the ER membrane and during this passage the signal sequence itself is cleaved from the peptide chain that follows it. Likewise, the addition of a particular carbohydrate core to polypeptides directs them to the Golgi complex, while mannose-6-phosphate is the signal which directs certain enzyme proteins to lysosomes. Many proteins e.g., proinsulin, also contain terminal sequences which are required for attaining a three dimensional structure. Such sequences are removed after folding of the polypeptide chain and bond formation of the disulphide bonds.

Structural-functional domains. Apart from its directing ability as stated above, the primary amino acid sequence of a protein contains information which determines the secondary, tertiary and quarternary structure of the protein molecule, enabling that molecule to carry out a specific cellular function. In many protein molecules, discrete structural domains or territories exist. These domains may represent a different functional part of the molecule. An example is the heavy chain polypeptide of the immunoglobulin molecule. It consists of four distinct structural domains, each of which corresponds to a different functional part of the immunoglobulin molecule.

It now appears that each exon of a eukaryotic gene codes for one structural-functional domain of a protein. For example, the heavy chain polypeptide gene for immunoglobulin contains four exons, each of which codes for a different structural domain of that polypeptide.

Existence of eukaryotic genes in pieces would allow for a more rapid evolution than would have been possible if the genes had no intervening sequences (introns). Since two pieces of DNA are more likely to recombine as the distance between them increases, genes with intervening sequences separating their exons could recombine more frequently to form new functional combinations than genes in a single piece. However, the presence of introns is not a necessity for gene expression in eukaryotic organisms, as shown by the absence of introns in the histone and the interferon genes.

Eukaryotic genes exist in pieces as a result of evolutionary changes which have made it possible to build functionally and structurally complex molecules from the earlier simpler ones.

Regulation of Eukaryotic gene expression

Recombinant DNA technology has shown that regulation of gene expression can occur at several of the points between DNA replication and protein synthesis.

DNA rearrangement

One form of genetic regulation, which controls cellular differentiation, involves alternatives in the structure of the main genes themselves, as for example in immunoglobulin synthesis. Early in development each antibody forming cell begins to produce only one type of light chain and one type of heavy chain out of a possible several thousands. The regulation of the production of both types of polypeptide chains involves structural rearrangement of the DNA coding for these polypeptides.

Chromatin structure

Many factors other than DNA rearrangements also have an influence on whether a given gene will be expressed or not. Chromatin containing actively transcribed DNA is much more sensitive to endonuclease digestion than chromatin which is not actively engaged in transcription. This may be due to variation in the types and amounts of non-histone chromosomal proteins bound to nucleosome cores and DNA linkers.

Histone and DNA modification

Certain non-histone chromosomal proteins are preferentially associated with actively transcribed genes. There is also an association beween selective histone regulation and gene expression.

A gene has also been shown to contain a lower proportion of methylated cytosine residues when it is actively transcribed, than when it is not expressed. Conversely, maintenance of X-inactivation may require methylation of certain sites on the X chromosome. However, factors playing a primary role in the regulation of gene expression are yet to be determined.

In addition to genes coding for proteins, there are also DNA sequences which code for structural RNAs, viz., ribosomal RNA and transfer RNA, which are actively involved in protein synthesis. Some small DNA sequences, in addition, code for other small RNAs, which may function in the nucleus as control elements. These RNA-coding genes also make up as much as one per cent of the total DNA despite the fact that such genes are very small in size and are only of a few types, the reason being that they occur usually in many copies each.

For any one type of cell or tissue, up to one per cent of the DNA is transcribed to messenger RNA coding for protein, and up to 10 per cent is transcribed to nuclear RNA. In different cells or tissues, many of the sequences are transcribed in all cells, perhaps owing to their universal importance, e.g., for some enzymes and cell membrane proteins; but other sequences appear to be unique to a particular cell or tissue. If embryonic tissues are also taken into account, it becomes obvious that a large proportion of the DNA is transcribed in the nuclei of some tissue cells or the other. For example, as much as 40 per cent of the total gene sequence in the nuclear DNA, in the embryonic rat brain, is transcribed into nuclear RNA.

Jumping Genes

One of the dogmas of Genetics was that the position or 'locus' of gene on a chromosome was fixed or static, and could only change either through mutation or through crossing over (recombination). It has been shown in bacteria and in **Drosophila** that genes and segments of DNA do move between and on chromosomes with frequencies as great as 10^2. These have been called 'jumping genes'. The heretical concept of jumping genes was first propagated in the early thirties by Barbara McClintock, the recipient of the 1983 Nobel Prize for Medicine and Physiology. Dr. McClintock, a botanist and plant geneticist, has worked life long on the Indian corn or maize plant. As far back as in 1951, she claimed that genes are not fixed on the chromosomes but can move around in a rather random manner. Her claim was entirely disbelieved and disregarded, much in the same way as was Mendel's claim based on his work on the pea plant. But for the results of the research of molecular biologists of the 1960s, which demonstrated that indeed genes are not fixed entities, her work would not have seen the light of day.

Dr. McClintock found that colour changes in the successive generations of corn did not follow predictable hereditary patterns. She concluded that genes which control the colour of a kernel and other similar characteristics could be activated or shut off by other genes acting as switches and that these switches could move from one part of a chromosome to another under the influence of yet another type of genetic element, which she called an activator.

Today, it is recognized that when bacteria develop resistance to an antibiotic, they can pass this trait to other bacteria by means of these jumping genes or transportable elements as McClintock has called them. Such genes may possibly play a part in the change of normal cells into cancer cells and even in speeding up the evolutionary process.

Overlapping Genes

The discovery that genes overlapped came by way of sequencing of proteins and nucleic acids; and was pioneered by Frederick Sanger in the fifties. It was then realized for the first time that the sequence and the number of amino acids in a protein were orderly and fixed. However, till then, no simple technique for knowing the base sequence of DNA was available. The development of the *plus* and *minus* method by Sanger and Coulson (1976) and lately of the chain terminator method by Sanger, Nickolson and Coulson (1977) provides an ingenious way of determining the base sequence of DNA, and is indeed a major landmark in molecular biology.

With these techniques, the entire genome of the phage ∅ × 174 (phi Ten 174) and phage G_4, have been sequenced. The virus ∅ × 174, a bacteriophage, is a **tailless icosahedron** (a solid figure having 20 faces or sides). Its diameter is 250A°, and it has spikes at its apices. Its **capsid** (or coat) is made up of an assortment of proteins. Its single-stranded and **circular genome** (DNA) is made up of approximately 5386 nucleotides.

Genetic mapping shows nine sequential genes A,B,C,D,E,J,F,G and H with the following functions: A—for DNA replication; B and D—for the assembly of phage particle; C—function not known; F,G,H—make structural proteins of the viral capsid (coat); J—codes for a small basic protein involved in the condensation and packaging of DNA; E—makes a substance for the lysis of the host *E. coli*.

A number of genetic tests indicated that gene E is completely included within gene D, i.e., the genes D and E utilize the *same* DNA sequence for protein synthesis but use different coding frames. Thus, gene D **overlaps** gene E. It was shown that gene E coincides with 60 per cent of gene D and lies towards its end. So, gene J becomes adjacent to gene. D. The end part of gene D was found to overlap the start of gene J. Also, gene B was found to be contained within gene A, so that they are both transcribed from the same DNA sequence. *Hence, each gene need not be a separate entity.*

A genome of around 5480 bases should have a maximum **coding capacity** equivalent of 1800 amino acid residues (5400 ÷ 3, a triplet for each amino acid). These amino acids would have a combined molecular weight of 200,000 daltons. In estimation, however, the combined weight of these proteins turned out to be much more, i.e., around 250,000 daltons. The new technique enabled Sanger *et al* to arrange amino acid sequences of some proteins of the phage alongside the base sequence of the corresponding genes and to their surprise they observed three facts:

1. The *same* gene was found coding for *two* different proteins.
2. One protein was coded for, in part, by one gene and *in part* by another, and
3. The **termination codon** for one gene was the **initiation codon** for the next contiguous gene. For example, the 360 nucleotide-long B gene is completely contained within the region of the 1536-nucleotide-long A gene, but is read in a different reading frame. Similarly, gene E of 273 nucleotides is contained entirely within a gene D of 456 nucleotides. Also, the terminator codon for gene D overlaps the initiator codon of the next contiguous gene J by one nucleotide. Similar overlapping genes have also been discovered in the virus G_4, also a single-stranded DNA virus that infects *E. coli*

Overlapping of genes obviously appears to be a compacting or space-saving device in minute organisms, where the quantity of DNA is small in relation to the number of proteins needed. But, unaccountably, overlapping is seen to occur even in larger organisms such as *E. coli:* In the tryptophan operon of *E. coli,* the terminator codon of the cistron A serves as the initiator codon for the next cistron B. Overlapping genes are thought to have evolved in viruses because they are under selective pressure to conserve a genome small in size.

Split Genes

The epoch making discovery of **overlapping** and **jumping genes** had not challenged the concept that the DNA sequence (cistron) needed for the synthesis of a particular polypeptide (or protein) was a continuous polynucleotide chain, so that a complementary, continuous, linear and functional sequence of mRNA could be transcribed. It would now appear that this DNA sequence *is not* present at one place, but in fact lies scattered in pieces in the chromosome. This means that bits of protein coding DNA are separated by sequences that do not code for the protein. Among the initial reports of this stunning find was one that came from Breathnack, Mandel and Chambon of the CNRS Laboratory for Eukaryotic Molecular Genetics at Strasbourg, France. In an attempt to isolate the chicken gene which codes for ovalbumin (protein of egg-white), the easily extractable messenger RNA (mRNA) carrying information for the structure of ovalbumin, was isolated. This mRNA was then subjected to the action of the enzyme *reverse transcriptase,* in the presence of radioactive tracers. The enzyme synthesised back the radioactive RNA sequence for ovalbumin, thus giving a copy of the radioactive gene ovalbumin mRNA. The radioactive DNA copy was incubated with the fragments of the original non-radioactive chromosomal DNA, which had the ovalbumin gene in one complete fragment. The two DNA fragments were expected to hybridize together. But the team was "shocked" to find radioactive DNA hybridizing with *three* quite different pieces of chromosomal DNA, instead of with one complete gene of ovalbumin. They knew that their enzyme could not have cut the ovalbumin gene and so "they were forced to the conclusion that the gene is split into three on the chromosome." So the "genes in mammals lie broken into pieces—and so do some of the basic assumptions of molecular biology."

Quite in contrast with gene-overlap as a device for gene compacting found in the **prokaryotes,** is the occurrence of split genes. **Split genes** appear to provide a method for accommodating more DNA than is necessary for the functioning of a gene. Whereas, prokaryotes require maximum coding capacity with minimum amount of DNA, the higher organisms or **eukaryotes** provide for a large amount of redundant DNA. A cell in a eukaryote contains about 1000 times more DNA than a prokaryote cell.

In eukaryotes, a gene is not made up of functionally contiguous units. Instead, in a single gene, there are five or more silent regions, the effects of which never appear in the final gene product. That is to say, the genes are split. These **silent regions** or **intervening sequences** are called **inserts.** They have now been termed **'introns'** to contrast with the functioning inter-intronic regions which are called exons. Exons could probably, and in rhyme with introns, be called 'extrons'. The introns seen so far range from 10 to 10,000 bases in length.

The **exon-intron concept** may serve to solve many a genetic riddle concerning mutation, recombination, and so on. Introns have been described as "frozen remnants of history" and "the sites of future evolution".

The concept of the **colinearity of genes** and their products got shaken during a study of the animal virus, adenovirus 2, at the Cold Spring Harbor Laboratory, New York. When the virus infects a nucleated cell, it is capable of making its own mRNA and other components by making use of the enzymes of the nucleated cell in which it multiplies. Therefore, the mRNA of these viruses has the characteristics typical of the eukaryotic mRNA, i.e., addition of 7-methyl guanosine at the 5' end also called 'capping', and polyadenylation which means addition of poly A tails to the 3' end.

In eukaryotes, the primary gene transcript is *a non-functional* precursor RNA and is called hetrogeneous nuclear RNA, or hnRNA. It can mature into the functional mRNA, only by a process of 'tailoring' at its 5' end and 3' end, as described above. The DNA of the adenovirus is a double-stranded linear molecule coding for about 20 proteins. Late in the life of the virus, eight of its genes are transcribed in large amounts (the 'late' genes). The transcript or transcribed product is a single large RNA fragment which gets cleaved into eight mRNAs. Peculiarly enough, the total length of these eight mRNAs which actually get translated into the various proteins is *shorter* than the original single transcript. When one of these 'late' genes, **the hexon gene,** was paired with its mRNA and the resulting duplex was examined with an electron microscope, it was noticed that while most of the duplex was paired, there were also unpaired regions which stood out as loops. this was the first ever *visual* indication that looping out could be a mechanism for excluding the extra DNA. Apparently the hexon mRNA was made by joining together *four different segments.* The insert sequences or introns are cleverly removed before the transcript goes into action as mRNA, and is ready to combine with the ribosomes. Split genes have also been encountered in several other viruses like the Rous sarcoma and murine leukaemia viruses. Apart from viruses, such genes have now been confirmed in the ribosomal genes of *Drosophila,* ovalbumin gene and ovomucoid gene of chickens, ß-globin gene of mice and rabbits, tRNA genes of yeast, and the immunoglobulin gene of mice.

The precursor mRNA (hnRNA) of all the genes studied so far contains **insert sequences.** This indicates that hnRNA is a faithful unaltered complementary transcript of the entire gene (including the inserts). In the production of active mRNA from this precursor, the inserts or introns get eliminated by the action of specific enzymes. The precise role of introns is under debate. Several possibilities have been projected, such as,

1. Introns regulate the **turning on, or off of genes.** This theory is being discarded.
2. Introns serve as **structural genes** or cistronic sites.
3. Looping out of introns is a simple device for discarding the **redundant DNA** which the cell had helplessly acquired during the course of its evolution.
4. According to Flavell, Glover and Jeffreys, inserts are a means of introducing **diversity** in gene sequences which gives a **selective advantage** to the organism.

DNA polymorphisms

The analytical techniques, viz., **restriction endonuclease analysis** and **Southern hybridization,** have revealed that not all restriction sites are identical on the DNA of all individuals. For instance, digestion of DNA from some individuals with the enzyme HpaI results in a 7.6 Kb fragment containing the human ß-globin gene (1000 bases = 1 kilobase = Kb). However, a similar digestion in other individuals who lack one of the HpaI restriction sites flanking the ß-globin gene, produces a 13 Kb ß-globin gene-containing fragment instead. Such **DNA polymorphisms** appear to be quite common in humans, and it seems likely that they will play an important role in human genetics in the future. The tight linkage of certain polymorphic DNA restriction sites with the ∝ and ß-globin genes in man has already provided a new approach to the prenatal diagnosis of disorders such as thalassemia and sickle-cell anaemia.

RNA-directed DNA synthesis

Contrary to the central dogma of unidirectional DNA-directed RNA-synthesis, Temin and Baltimore suggested from viral studies that genetic information could also flow in a reverse direction i.e. from RNA to DNA to effect RNA-directed DNA synthesis. Parts of DNA in a normal cell serve as templates for the synthesis of RNA which in turn acts as a template for the DNA synthesis. The DNA gets incorporated with the cellular DNA and enlarges it. Temin has postulated that the resultant enlargement of some part of DNA may be an important factor in embryonic differentiation and possibly in cancerogenesis.

Mutation

Mutation is any sudden heritable structural change in DNA. It also refers to the process by which a gene undergoes a structural change. The term mutant is used for a modified gene resulting from mutation. By extension, it may refer to the individual manifesting the mutation.

Mutations may be subdivided according to:

A. **Cause of mutation.**
   (1) spontaneous
   (2) induced by exogenous agents

B. **Type of change brought about by a mutation.**
   (1) genome mutations (numerical chromosomal aberrations)
   (2) chromosome mutations (structural chromosomal aberrations)
   (3) gene or point mutation (alteration in the DNA at the molecular level)

C. **Place at which a mutation occurs.**
   (1) somatic mutations (occurring in body cells)
   (2) germ cell mutations (occurring in germ cells—gametes)

Family patterns of disease due to mutant genes of severe (large) effect:

The diseases due to **mutant genes** can be divided into **dominant, recessive and X-linked.** It has been suggested that the polypeptide products of the genes involved in dominant conditions make up structural proteins, whereas those concerned in recessive conditions make up enzymes. The X-linked conditions are determined by mutant genes located on the X chromosome.

Dominant conditions. A characteristic pedigree would show that the first case in the family appears **sporadically** as a result of **fresh mutation.** An affected child is born to normal parents and has normal siblings. Thereafter there is an average 1:2 risk to the offspring of those affected. Examples are von Willebrand's disease, hypokalaemic periodic paralysis, and Huntington's chorea.

When the condition reduces fitness to zero, (i.e., patient begets no children) all cases would be sporadic, i.e., they would have no similarly affected relatives, e.g., Apert's type of acrocephalosyndactyly.

Recessive conditions. The family pattern of recessive conditions commonly shows sibs of affected person to be also affected but it is unusual for anyone else in the family, including half-sibs and offsprings to be affected. Another feature of the pedigree of recessive conditions is a raised frequency of first cousin or other **consanguineous marriages** among the parents of the patients.

X-linked conditions. The typical family pattern of X-linked conditions depends on the fact that women heterozygous for the mutant gene are likely to transmit the disease to half their sons; and half their daughters are likely to be heterozygotes **(carriers).** When a man survives and has children, all his daughters are heterozygotes, but none of his sons are affected and none of them can transmit the mutant gene. A substantial proportion of patients with severe X-linked conditions appear as sporadic cases. In some instances the mother is not a heterozygote but mutation occurs during the formation of her ova.

Spontaneous mutation

The **spontaneous mutation rate** varies for different loci, the limits of the observed range being between one in 10,000 (1×10^{-4}) and one in 1,000,000 (1×10^{-6}) per locus per gamete, with an average of about 1×10^{-5}. Some loci, especially the **blood group loci,** are virtually not observed to mutate. At the opposite end of the range, the highest mutation rates measured in man are those of neurofibromatosis and Duchenne muscular dystrophy, both of which are in the range of 10^{-4}. Assessment of mutation rates and conditions modifying them is one of the main fields of **mutation research** in humans.

For rare dominant mutations, the method of measurement is relatively easy.

$$\mu = \frac{n}{2N}$$

Where, μ = rate of mutation, the
n = number of cases of the disorder that

have been born to normal parents in a defined area over a defined time span and

N = Total number of births in the same area during the same period.

Since a new dominant trait required a mutation in only one of the two gametes, the factor ½ is involved in calculation.

This is the direct method of estimating mutation rate. However, for accurate measurement, care should be taken to exclude **genetic heterogeneity,** i.e., whether the same phenotype is produced by mutation at more than one locus or by environmental factors.

It is difficult to determine the mutation rate of recessive genes and of X-linked genes. In recessive genes there is no demonstrable heterozygous expression. For X-linked genes, it is not possible to know whether a sporadic case of a disorder has been inherited from a heterozygous but phenotypically normal mother or whether it represents a new mutation.

Factors influencing spontaneous mutation rates

Parental age and sex: Most mutations involving numerical aberrations of chromosomes are caused by **non-disjunction** during the first or second meiotic divisions. Non-disjunction is more common in female than in male germ cells; roughly 2/3 of all trisomies are due to **female non-disjunction.** Moreover, the **risk of non-disjunction** increases with increasing maternal age, especially for women above 35. A certain increase for older men is also observed. This has been demonstrated for fathers of children with **new dominant mutations.** Fathers of new-mutant achondroplastic dwarfs are on an average a few years older than other fathers in the population.

Other factors influencing the non-disjunction rate appear to be **autoimmune processes** and possibly an increased tendency for **satellite association** among acrocentric chromosomes.

Gene or point mutation (Fig. 50): Alteration in the DNA at molecular level: Molecular mechanisms of mutation

Base substitution

There are many types of gene mutation. The most common are true mutations involving **single base substitutions** in just one triplet of bases in a structural gene which may be over 100 triplets of bases long. Most of the abnormal haemoglobins are examples of such mutations. In these haemoglobins, there is just one amino acid substitution in a known position in either the ∝ or the ß peptide chain. In sickle cell haemoglobin (haemoglobin S) valine is substituted for glutamic acid in the sixth position of the ß peptide chain. This is brought about by a change from GAA (or GAG) to GUA (or GUG) mRNA codons. In haemoglobin C, lysine replaces glutamic acid, again in the sixth position of ß peptide chain. Here, GAA (or GAG) is changed to AAA (or AAG).

Hundreds of other abnormal variants of haemoglobin are known, some of the ∝ chain and some of the ß chain, which result in amino acid substitutions in this way. The clinical effect of such amino acid substitutions varies from no apparent effect to a completely unstable haemoglobin molecule and all its consequences.

When a purine base (adenine or guanine) is substituted for a pyrimidine base (thymine or cytosine), this type of substitution is called **transversion.** Substitution of one purine base for another purine base or a pyrimidine base for another pyrimidine base is known as **transition.**

The single base substitutions in the coding DNA strand are **point mutations** in the strictest sense of the word. These point mutations are distributed more or less randomly over the entire gene. Their phenotypic effects differ depending on the location and kind of the resulting base substitution.

At times a single base substitution leads to a stop codon. In this case, the gene is not transcribed beyond this point. The contrary has also been observed, the original stop codon mutating to give a codon for a specific amino acid. Then, the DNA strand is transcribed up to the next stop codon. The result is a **chain elongation mutant.**

Loss of base pairs

The other mode of origin of mutation is loss of base pairs from the DNA strand. As shown in Fig. 50, elimination of one or two base pairs leads to a **frameshift mutation.** The amino acid sequence 'downstream' from the mutation becomes entirely different. If the number of bases eliminated happens to be three or exact multiples of three, the effect on transcription is less drastic since one or only a few amino acids are lacking in the molecule.

When a peptide chain forms a part of an enzyme, the effect of gene mutation may vary from some alteration in enzyme specificity to complete loss of enzyme activity. Under such circumstances, the heterozygote is usually normal clinically as is the case with heterozygotes for the gene responsible for phenylketonuria, galactosemia, Hurler's type of mucopolysaccharidosis, and many other inborn errors of metabolism. There is a considerable safety margin with most enzymes, and though the heterozygote will often have little more than 50 per cent of normal enzyme activity; there may be no clinical manifestations. The **mutant homozygote** with no normal gene at the locus concerned, shows severe deficiency in enzyme activity and is clinically affected. When gene mutation produes clinical effects in a heterozygote, it is likely that the peptide chain synthesized by the gene is a constituent part of the structural organization of the cell.

Examples of mutations involving operator or regulator genes are not known in man. The effect of such mutations would be a quantitative alteration in the production of normal peptides. The ∝ and ß thalassemias could be such instances.

Abnormal crossing over (Lepore-type mutants)

When unequal crossing over takes place between two closely placed loci, it results in abnormal recombination and is referred to as Lepore-type mutants. This is exemplified by **Haemoglobin Lepore** which is the result of unequal crossing over between two closely linked and very similar genes coding for ß and polypeptides. As a result thereof the mutant codes for non-∝chain having both the N-terminal portion of a normal and the C-terminal portion of a normal ß chain.

Abnormal crossing over (gene duplication)

One type of mutation that can occur is gene duplication. It may occur by unequal crossing over and perhaps by duplication during copying of DNA. Either process gives two similar gene-loci in adjacent positions on a chromosome. Haptoglobin alleles offer an example of the origin of mutant alleles by "unequal" crossing over. (Fig. 51). Haptoglobins or ∝ globulins are types of serum protein, which are concerned with the binding of haemoglobin from aged and broken-down red blood cells. The haptoglobin molecules are composed of two kinds of polypeptide chains, ∝ and ß (not related to the ∝ and ß chains of haemoglobin). A number of different haptoglobins are found in different individuals. The differences in haptoglobins are due to variations in the ∝ chains, the ß chains being everywhere the same.

Three alleles at the *Hp* (haptoglobin) locus are responsible for the three main kinds of ∝ chains found in human populations. Two of the alleles are Hp^{1F} and Hp^{1S}. They control ∝ chains that differ from each other by a single specific replacement of one amino acid. The third allele, Hp^2 differs from both Hp^1 alleles in being nearly twice as long i.e., a combination of two nearly complete Hp^1 chains arranged in tandem sequence. The presumed origin of such an allele is shown in Fig. 51. It is likely that a Hp gene of normal length underwent crossing over with its allele in the

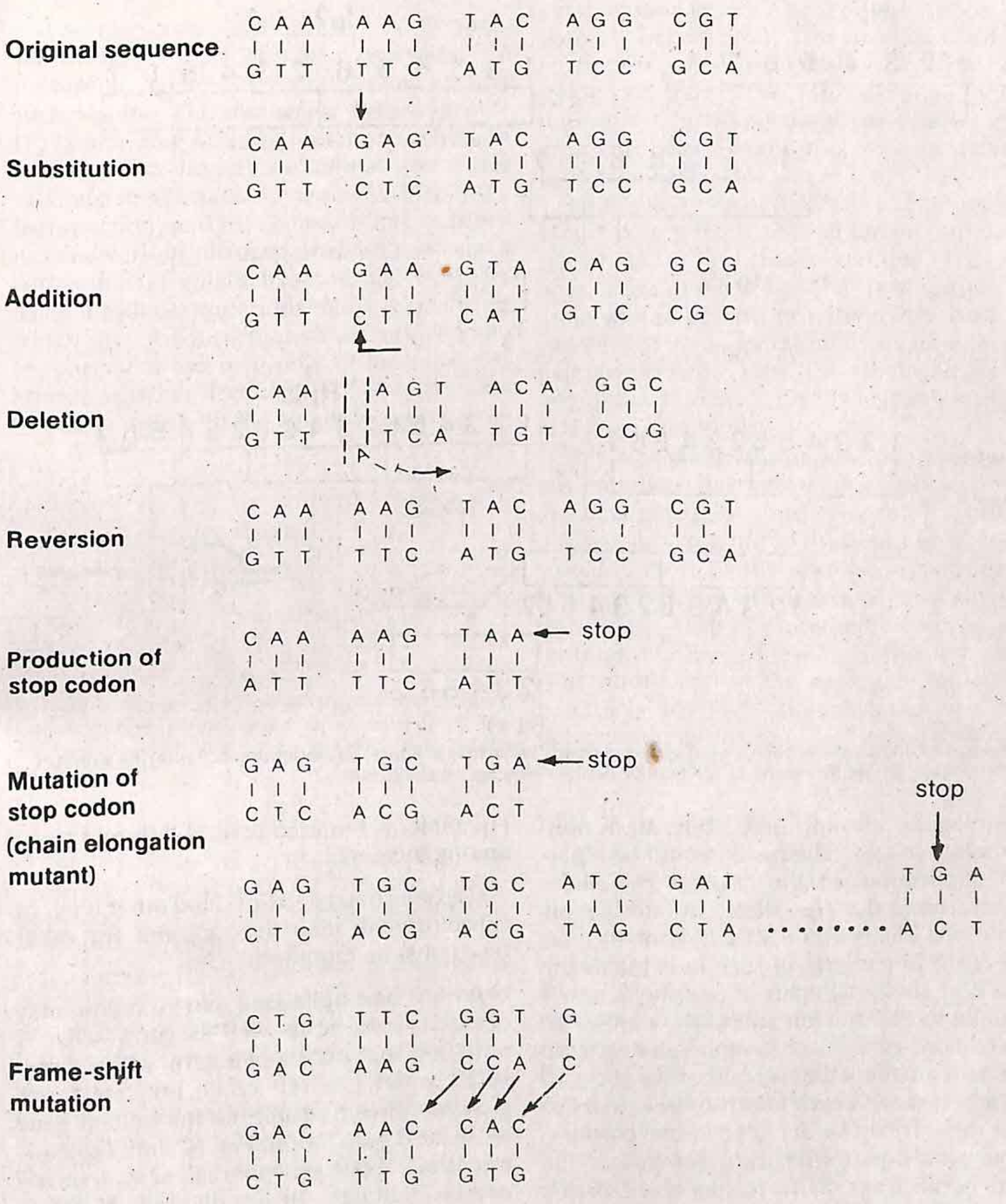

Fig. 50

Schematic representation of different kinds of point mutation.

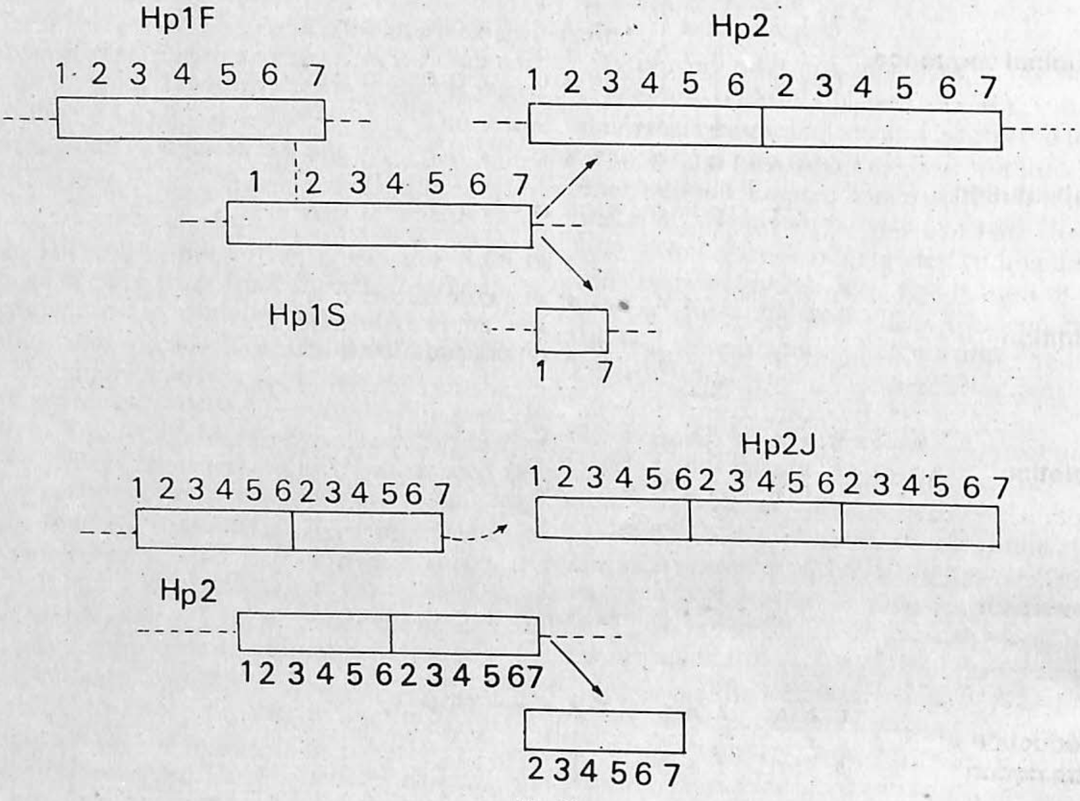

Fig. 51
Presumed origins of the haptoglobin alleles of nearly twice (upper figure) and thrice (lower figure) the length of the original genes by means of unequal or nonhomologous crossing over.

homologous chromosome, but at a nonhomologous site. The result would be a partial duplication, of the original *Hp* allele, thus forming the Hp^2 allele. In later generations individuals would arise as homozygotes for Hp^2 . In gametes of such individuals the two Hp^2 alleles can pair in two ways, either equally so that the left half of the duplicated gene in one chromosome would pair with the left half of the other and likewise the two right halves with each other, or unequally so that the left half of the gene in one chromosome would pair with the right half of the gene in the other. In the former case crossing over within the paired section would not alter the alleles but in the latter, an almost triplicated gene Hp^{2J} would arise as well as a reverted allele of single length. Hp^{2J}, a rare Hp allele, is found in several different types among humans.

Chromosomal deletions and other types of chromosomal anomalies account for other types of gene mutation.

It must be emphasized that mutations may occur in somatic as well as germ cells. A mutation that occurs in a germ cell is discovered when this cell or its progeny forms gametes, thus transmitting the mutant gene to the next generation. Fig. 52 shows somatic mutation. If one gene in a cell of a very early embryo mutates, further divisions of unmutated and mutated cells would build up in an individual whose tissues would comprise partly of mutated and partly of unmutated cells.

Induced Mutations

The mutational change may occur **spontaneously**, i.e., without exposure to mutagenic agents, or it may be induced by such agents. The **mutagenic agents** include (a) penetrating ionizing radiation such as X-rays, gamma rays from radium and other radioactive substances, neutrons from nuclear reaction, and (b) chemicals like mustard gas and various nitrogen or sulphur-mustard compounds, formaldehyde, nitrous acid etc. They produce mutations only in cells on which they act directly and are not specific for particular loci or groups of loci. It is not known whether frequency of spontaneous mutation rises with the age of a person.

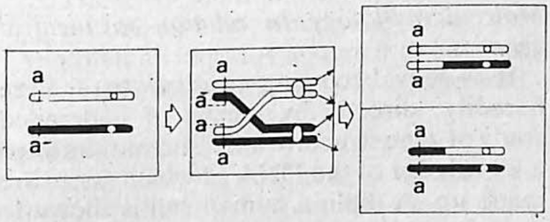

Fig. 52

Somatic mutation. a cell of genotype $a\ a^-$ undergoing mitosis, involving crossing over of alleles a and a^-. The resulting daughter cells have a genotype $a\ a$ and a^-a respectively.

Load of Mutations or Mutational Load

The load of genetic damage in man is not accurately known, but it is likely that at least 6 per cent of all persons born have some tangible genetic defect.

It is very difficult to know how many of these mutations are new and how many have been inherited from the previous generation. It has been estimated that each individual in the population carries three to five **lethal equivalents,** a lethal equivalent being defined as one gene which, if homozygous would be lethal or two genes which if homozygous, would be lethal in half the homozygous persons, and so on.

If a gene in the heterozygote state is advantageous, it is preserved and increased in proportion in a population, even if that very same gene when homozygous, is severely detrimental. The classical example is the resistance to malaria offered by the sickle-cell trait. HbC, thalassemia, G6PD deficiency and Fy allele of the Duffy blood group are also thought to provide protection against malaria.

Mutation is one of the basic phenomena of life. It is very likely that all life on our globe goes back to one common origin. It could have developed from the first group of molecules in which a primitive, information-carrying unit cooperated with an energy-gaining device, into the multiplicity of organisms of today, only through many ever-recurring mutations.

It is reasonable to suppose that **spontaneous mutations** have been taking place, pouring new genes into the gene pool continuously ever since life existed on earth. Individually, most of the mutations cause little harm; in fact they are **beneficial,** providing as they do the **basis of evolution**. However, the harmful mutant genes, accumulated over generations and in the aggregate, either in heterozygotes or homozygotes or in polygenic combinations, decrease the **fitness** of the population.

DNA Repair (Fig. 53)

DNA aberrations arise by exposure to ultra-violet rays or radiations or from internal cellular events whose origins are as yet not known. There are mechanisms prevalent in all forms of life to repair the **damaged DNA.** Evidence for the presence of **DNA repair mechanisms** is obtained from serious consequences caused by malfunctioning of the same. The rare human autosomal recessive disease **xeroderma pigmentosum** is accompanied by extreme sensitivity of the skin to ultra-violet light and increased incidence of skin cancer. It has been shown that the cells of such affected individuals are unable to repair DNA damage induced by ultra-violet light. The defect in this case appears to lie in the inability of an endonuclease enzyme to make the original cut that initiates the **excision-repair process.** In patients suffering from premature aging—progeria—the person usually has a defect of the ligase enzyme which is necessary to rejoin broken DNA strands.

When DNA is exposed to ultra-violet light or irradiation, the DNA molecule gets damaged. There now appear thymine, thymine-cytosine, or cytosine dimers (pyrimidine dimers) in the DNA molecule. The repair begins with a **special endonuclease** enzyme producing a cut in the phosphate-sugar backbone at a nucleotide position adjacent to the thymine dimer. The 3′ phosphate group at this point is then removed by another enzyme phosphatase or 3′ exonuclease leaving a nucleotide end bearing a 3′ hydroxyl group. A 5′ exonuclease then removes a 6 or 7 nucleotide-long-section including the thymine dimer. The deleted portion of the excised strand is then refilled by the DNA polymerase enzyme in the 3′-5′ direction, using the complementary strand as a template. The 3′-5′ gap that remains between adjacent nucleotides after polymerase activity is closed by a joining enzyme called polynucleotide ligase.

DNA damage brought about by ultra-violet rays is undone by **photoreactivation mechanism.** In this repair mechanism, the ultra-violet induced damage is reversed by exposing the cells to light containing visible wavelengths in the blue spectrum. The photoreactivation mechanism indicates that the damage caused by ultra-violet light can be reversed before the genetic material is permanently affected. In photoreactivation, the photoreactivation enzyme locates itself on the DNA molecule at the point where pyrimidine dimers are formed. Absorption of visible light radiation in the blue end of the spectrum provides the energy, which enables the enzyme to split the dimer. The DNA molecule then resumes its normal shape and the enzyme is released.

Molecular Biology in relation to medical genetics

It is now becoming possible to analyse heredity, directly by means of a detailed study of gene structure and function, through a knowledge of the DNA of which genes are made up. A diploid human cell is shown to contain a staggering quantity of DNA, viz., a total of 6×10^9 base pairs, or a molecular weight of 3.6×10^{12} daltons (2.8 picograms). Hypothetically, this amount of DNA would be enough to specify the sequence of about 5

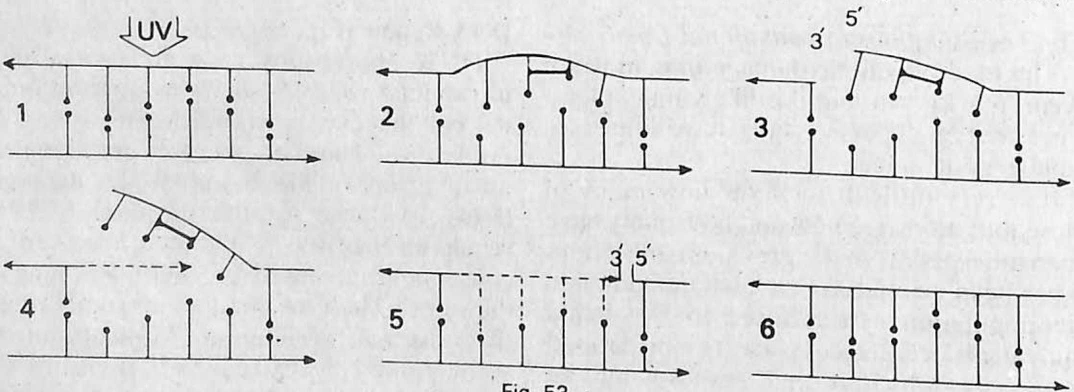

Fig. 53

A schematic representation of DNA repair, following its damage by ultra-violet radiation.

1. Damage of DNA strand by ultraviolet light.
2. Formation of thymine/cytosine/thymine-cytosine dimer.
3. A special endonuclease enzyme cleaves the DNA strand adjacent to the dimer.
4. Synthesis of a new DNA strand begins, using the intact strand as a template by DNA polymerase in 3′ - 5′ direction.
5. Complete excision of the damaged strand by an exonuclease.
6. The 3′ - 5′ gap that remains between adjacent nucleotides after polymerase activity is closed by polynucleotide ligase, the joining enzyme.

million different polypeptides, if all proteins had the size of globin, and if every gene were to code as globin genes do.

Various techniques place the current estimates of the number of coding genes at about 20,000 or one per cent of the total nuclear DNA. These estimates are based on the finding that 20,000 different cell proteins can be identified and on the assumption that one gene codes for one protein. The methods used to arrive at these estimates are:

1. Use of two-dimensional gels for direct protein identification
2. A calculation of the mRNAs by biophysical annealing to radioactive complementary DNA (cDNA) and
3. The extrapolation from organisms such as *Drosophila,* where the number of polytene bands is assumed to be closer to the number of proteins synthesised.

Gene Libraries

A gene library is a collection, complete or partial, of the cloned gene sequences from the DNA of an individual or a species. A genomic library contains sequences of nuclear DNA. A cDNA library, on the other hand, contains copies of the expressed messenger RNA.

Partial libraries can be obtained by cloning various fractions of DNA, e.g., by size, by base composition or by rate of reannealing. Libraries of mitochondrial DNA, comprise of DNA specifying one type of RNA or of DNA which appears to be actively transcribing RNA.

A genomic library would have enough recombinant clones, so that every DNA sequence in the nuclear genome is represented at least once. Therefore, every coding gene, regulatory element or repeated sequence, would be present. This is the basic source for specific gene isolation, since each DNA sequence is permanently available in pure form in unlimited amount, once cloned.

Another crucial role of gene libraries, in human genetics, is that such a library containing the total genome of a given individual in recombinant form, would enable a comparison of any given gene from this individual with a similar gene from any other individual.

DEVELOPMENTAL GENETICS

This branch of genetics deals with the mechanisms which activate or inactivate certain genes at appropriate times during embryogenesis, and foetal and postnatal life. All the cells of a multicellular organism have the same set of genes as the zygote from which they are derived, yet each cell type differs greatly from other differentiated cells. At a given time only a part of the total genome remains active and it is the active region that determines the nature of differentiation. Differentiation does not depend on differences in the genes present, but it depends on the variations in the activity of the genes.

Experimental evidences for genetic control in development:

1. If a nucleus from the intestinal epithelium of a tadpole is introduced into an anucleated unfertilized egg, the egg can develop into a normal adult frog.

2. The enzyme cystathionine synthetase is normally synthesized by the liver but not by skin cells. However, in tissue culture, skin fibroblasts become capable of synthesizing this enzyme.

Points 1 and 2 show that all genes are retained in all cells and that the genes remain inactive in the cells when their activity is not needed. The process of **cell specialization** and **differentiation** involves the **differential activity of genes** present in all cells, rather than the selective elimination of unwanted genes.

3. In amphibian eggs, the ribosomes used in the early part of embryonic development are those manufactured during oogenesis; and no new ribosomes are synthesized till the gastrula stage of development.

4. When the nucleus from an adult

neurone is transplanted into the cytoplasm of an anucleated unfertilized egg, the transplanted nucleus adopts the functions typical of the normal host cell nucleus.

Points 3 and 4 show that the egg cytoplasm is capable of influencing the activity of genes in the nucleus.

5. The chromosomes in the salivary glands of certain insects are enlarged and have distinctive patterns of transverse bands representing sites of different genes. During larval development certain bands swell and produce "puffs" in a characteristic sequence at certain sites on specific chromosomes. Puffing is associated with the synthesis of RNA. It is suggested that **puffs** are derepressed (activated) genes. A puff in one region and its absence at another illustrate the sequential **derepression** and **repression** of genetic loci during development.

6. Ecdysone, a hormone produced by the prothoracic glands of insect larvae, is responsible for moulting of the larvae. **Ecdysone** injected into young larvae induces the same sequence of puffing which occurs during larval development. This is an instance of a chemical substance bringing about derepression. In humans, random inactivation of the X chromosome (Lyon hypothesis) probably represents a similar mechanism of repression-derepression.

7. The phenomenon of **embryonic induction**, where one tissue determines the fate of another—e.g., ability of the chordamesoderm to induce the overlying ectoderm to differentiate into neuroectoderm—is brought about by a chemical agent called the inducer. Experiments to study the nature of the inducer and the process of induction, suggest that the substance which diffuses out of chordamesoderm and acts as an inducer on the ectoderm contains RNA. The process of induction is due to the gene product of one cell diffusing out of that cell and then inducing changes in the adjacent cells. It seems that the active principle in embryonic induction is a nucleoprotein.

8. In a *dwarf* mouse the mutant gene—recessive in homozygous state—brings about growth retardation, because of a poorly developed pituitary. Implantation of normal pituitary cells restores the mouse to normal size. This illustrates the influence of a hormone on growth and development.

9. If a *pygmy* mouse has a mutant gene, implantation of normal pituitary does not restore the normal growth. Pituitary cells from pygmy mice are effective in restoring normal growth in dwarf mice. This means that the *pygmy* mutant produces sufficient growth hormone, but its tissues are refractory to the hormonal effects.

In man, comparable conditions due either to defective synthesis of the growth hormone or to a peripheral unresponsiveness to the growth hormone are seen.

10. In *Drosophila,* several lethal mutants have been studied, e.g., *lethal giant larvae, meander and translucida.* Each of these **mutant genes** alters the normal course of events at a **specific time in development.** This illustrates that there are lethal mutants which interfere with normal development resulting in death during development. It also suggests that the normal alleles of these mutants bring about normal development. During normal embryogenesis, the developmental changes are a result of many genes acting in an orderly sequence and any disturbance in that activity, either because of genetic mutation or because of environmental agents (e.g. radiation) known as **teratogens,** can result in **congenital malformations.**

11. It has been shown that if chromosomal DNA is freed of its histone, the amount of RNA synthesis goes up. This suggests that in the intact chromosome, histones inhibit DNA-dependent RNA synthesis.

12. Many enzymes are shown to exist in multiple molecular forms known as isozymes or **isoenzymes,** e.g. lactic dehydrogenase isozyme patterns show progressive changes during foetal and postnatal development.

Similarly the change from foetal to adult haemoglobin during late foetal and early postnatal life (Fig. 65, 66) reflects changes in the activity of specific genes.

Based on these observations, it has been suggested that the sequence of events during embryonic development is a result of **genetic switches,** i.e., a particular effector substance sets in a sequence of developmental processes by switching on or off certain genes. Repressed genes get activated if they are responsive to the effector. This may bring about switching on to new developmental pathways. Some derepressed genes may produce enzymes responsible for changes brought about in the metabolism of adjacent cells and tissues.

SUMMARY

Molecular genetics: Deals with DNA and RNA, replication of DNA, its transcription and translation into polypeptide chains – the basis of proteins.

DNA: Structure: A helically coiled, double-stranded molecule consisting of linearly arranged nucleotides. A nucleotide is made up of a nitrogenous base – either a purine (adenine or guanine) or pyrimidine (thymine or cytosine) – a deoxyribose pentose sugar and a phosphate radical.

RNA: Structure: A product of DNA, single-stranded, made of nucleotides – a nitrogenous base – either a purine (adenine or guanine) or pyrimidine (uracil or cytosine), a ribose pentose sugar and a phosphate radical.

Types of RNA: 1. Messenger or mRNA. 2. Ribosomal or rRNA and 3. Soluble or transfer or tRNA.

Gene: Made up of varying lengths of DNA.

Genetic code: Codon: A sequence of three successive base pairs (triplets) along DNA strand coding for one amino acid. Sixty-four possible codon arrangements specify the 20 different amino acids found in proteins. This, so-called genetic code, is said to be universal, 'non-overlapping' and 'degenerate'. One specific codon initiates protein synthesis. Three of the codons do not code for any amino acid.

Use of techniques such as restriction endonucleas analysis, recombinant DNA cloning and DNA sequencing has resulted in revision of the earlier ideas of eukaryotic gene structure. Gene is now shown to exist in pieces such that the DNA sequences which code for a particular polypeptide—the exons' are interrupted by one or more sequences of DNA – the 'introns' which do not code for a polypeptide.

Existence of eukaryotic genes in pieces presumably allows a more rapid evolution than would have been possible if the genes had no intervening 'noncoding' sequences – the introns.

Protein synthesis: DNA directs the synthesis of protein through the agency of messenger RNA and ribosomes.

Mutation: It is any sudden heritable structural change in DNA.

Classification:

1. According to the cause, (a) spontaneous; (b) induced by exogenous agents.
2. According to type of change (a) genome mutations (numerical chromosomal aberrations) (b) chromosome mutations (structural aberrations in chromosomes) (c) gene or point mutation (alteration in DNA at the molecular level).
3. According to site of mutation (a) somatic (occurring in body cells) (b) germ cell mutations.

Extraneous agents which induce mutations are called mutagenic agents and include, (a) ionizing radiations like X-rays, (b) chemicals like mustard gas, formaldehyde etc., and (c) some viruses.

Spontaneous mutations, presumably occurring continuously since the inception of life on earth are the source of new genes which have provided a basis for evolution. Individually, a mutation may cause little harm, but an accumulation of a number of harmful mutant genes over several generations and in the aggregate would tend to decrease biological fitness of a population.

Developmental genetics studies the mechanisms involved in cell proliferation and cell differentiation which result from 'differential' gene activity, both in space and in time to give rise to an embryo from a zygote.

It has been suggested that the sequence of events in embryonic development is a result of 'genetic switches'. A gene product or 'effector substance' could possibly set in a sequence of developmental processes by switching on or off certain genes.

QUESTIONS

1. Give the definitions of the term 'gene', from various points of view.
2. Write notes on:
   DNA
   RNA
   mRNA
   tRNA
   rRNA

Codon
Nucleotide
Nucleoside
Nucleosome

3. Describe the molecular structure of a gene.
4. Describe and discuss the genetic code.
5. Write notes on:

Genotype
Phenotype
Mutation
Role of DNA and RNA in protein synthesis

6. Write notes on:

Exon
Intron

5 MODES OF INHERITANCE

INTRODUCTION

Modes of inheritance describe the different ways in which genes handed down from parents to their offspring through several generations, may express themselves. Genes exist in pairs on **homologous chromosomes,** except on the X and Y chromosomes. Even on the X and the Y chromosomes, there is a possibility of a few loci carrying **homologous** or **allelomorphic genes.**

An inherited trait, e.g., a physical feature, a blood group, or an enzyme system may depend on a single gene pair or on the cumulative effect of a large number of genes. The former is called **Mendelian** or **unit factor inheritance** and the latter, **polygenic inheritance.** Mendelian inheritance may be either **autosomal** or **sex-linked.** In autosomal traits, the genes are located on any of the 22 pairs of autosomes and in sex-linked traits, the genes belong to the X or Y chromosome.

An inheritance is called **autosomal dominant,** when one member of an allelic pair is able to express itself in total disregard to the presence of the other member. On the other hand, **autosomal recessive inheritance** depends on the expression of both partners of an **allelic pair.** When the two members of an allelic pair are identical, they are said to be **homozygous** and when they are unlike each other, the combination is said to be **heterozygous.** Thus a **dominant gene** expresses itself, both in its homozygous state and in its heterozygous state, while a recessive gene expresses itself only in its homozygous state.

A trait is said to constitute the **phenotype** of an individual, while the allelic pair of genes determining the trait constitute the **genotype** for that trait.

When both members of an allelic pair are able to express themselves fully in the phenotype, the inheritance is called **codominant,** e.g., the ABO blood group. Sometimes, a trait is the result of a sharing of, or a partial expression of both alleles. It is then called **intermediate inheritance,** e.g., sickle-cell trait.

The Y-linked Mendelian traits can only find expression in the male line and hence are often called holandric. The incidence of X-linked traits on the other hand, differs markedly in the females and the males, since the female karyotype is XX and the male, XY. Thus, X-linked recessive disorders find a ready expression in the male, since the so-called recessive allele is not matched by any counterpart on the Y chromosome. On the other hand the X-linked recessive gene is not ordinarily expressed in the female, since the accompanying dominant allele prevents its expression.

Mendelizing genes produce a readily recognizable qualitatively varying trait, whereas by contrast, in polygenic inheritance, the effects are quantitatively varying and require large scale measurements and plotting of curves to assess their effects. However, even in traits depending on single gene pairs, the expression may be highly variable in view of several environmental factors.

The primary effect of a gene pair may be far removed from its phenotypic effect, which may consist of several apparently unrelated signs and symptoms. This phenomenon is referred to as **pleiotropism** or **pleiotropy.** In sharp contrast with pleiotropy is the phenomenon called genetic heterogeneity, wherein, the same phenotypic effect in different members of a population, may result from independent activity of several genes.

* * *

Genes carried on chromosomes are responsible for the development of inherited characters or traits. When a single gene pair determines a trait, the trait is known as single-gene trait and its mode of inheritance from one generation to the next generation follows Mendel's law of unit inheritance and segregation. When many genes determine a trait, the trait is known as polygenic and it follows the pattern of polygenic inheritance for transmission from generation to generation.

The terms defined below are used in genetics to convey special meanings with reference to inheritance.

Allele or allelomorph. One or more alternative forms of a gene found at the same (corresponding) locus on homologous chromosomes in an individual and/or in a population.

Homologous genes. Identical alleles occupying the same locus on homologous chromosomes.

Homozygous. An individual (homozygote) who possesses two identical alleles at one particular locus on homologous chromosomes.

Heterozygous. An individual (hetrozygote) who possesses two different alleles at one particular locus on homologous chromosomes.

Compound. A heterozygous individual who possesses two different mutant (not 'wild or normal') alleles at one particular locus on homologous chromosomes.

The three terms, homozygous, heterozygous and compound are used for an individual as well as for his/her genotype.

Dominant. An allele which is always expressed, both in homozygous and heterozygous combinations. A trait which is expressed in individuals who are homozygous or heterozygous for a particular gene, is also said to be dominant.

Recessive. An allele which is expressed only when it is homozygous. It also refers to a trait which is only expressed in homozygotes for a particular gene, and not in those who are heterozygous for that gene.

Genotype. The total genetic constitution (genome) of an individual, or more specifically the alleles present at one locus, for a particular trait.

Phenotype. The appearance (physical, biochemical, and physiological) of an individual; produced by expression of the genotype under the influence of environment.

The study of a particular trait in a family usually begins with the person first found to exhibit the trait and through whom the family draws the attention of the investigator. Such a person is referred to as the **propositus** when a male and **proposita** when a female, (also called **proband** or **index case**). The data collected from a family over a number of generations can be represented, in a chart using international conventional symbols (Fig. 54). Such charts are known as **pedigree charts.**

SINGLE-GENE TRAIT INHERITANCE

Many genetic traits are determined by genes at a single locus; such traits may be dominant or recessive. Nearly 3000 such conditions, most of which are abnormalities rather than variants of the normal, are known to be single-gene traits. The vast majority are serious, none are curable and relatively few are treatable or manageable.

There are 1364 disorders, where the mode of inheritance has been firmly established. These include 736 **autosomal dominant**, 521 **autosomal recessive** and 107 **X-linked disorders.** There are further 1447 disorders where **unifactorial inheritance** is suspected, but not yet proven.

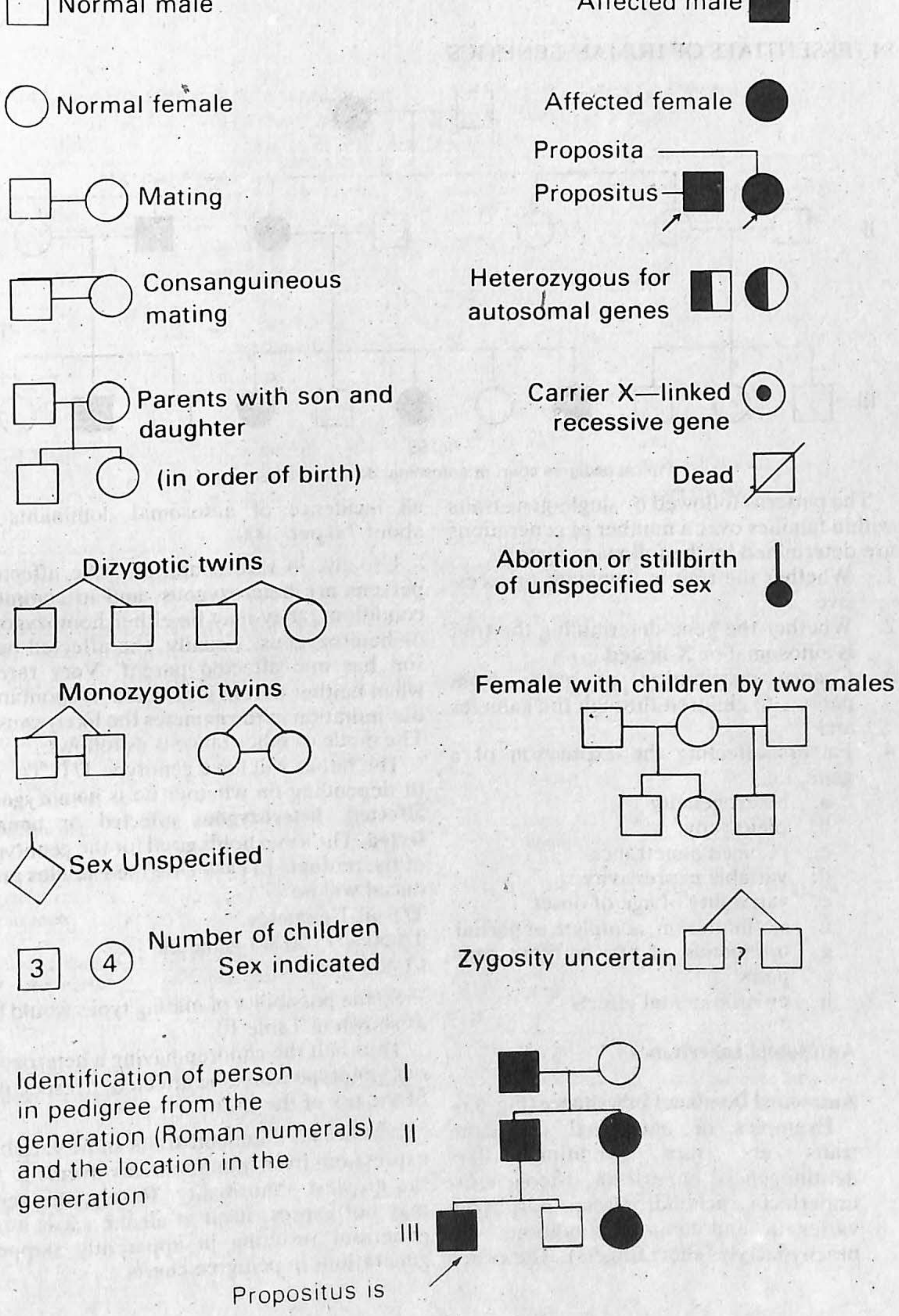
Normal male
Affected male
Normal female
Affected female
Proposita
Propositus
Mating
Consanguineous mating
Heterozygous for autosomal genes
Parents with son and daughter
(in order of birth)
Carrier X—linked recessive gene
Dead
Dizygotic twins
Abortion or stillbirth of unspecified sex
Monozygotic twins
Female with children by two males
Sex Unspecified
Number of children
Sex indicated
3
4
Zygosity uncertain
?
Identification of person in pedigree from the generation (Roman numerals) and the location in the generation
I
II
III
Propositus is

Fig. 54

Chart showing the commonly used genetic symbols.

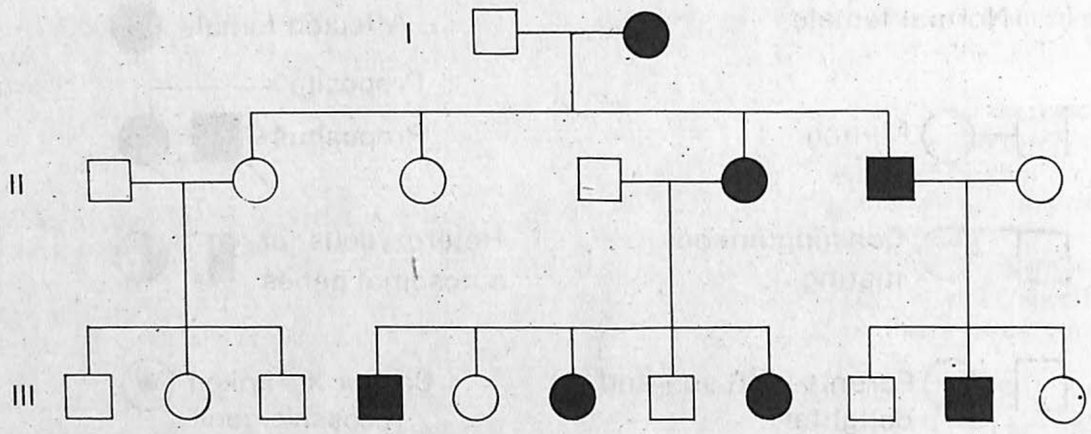

Fig. 55

Typical pedigree chart of autosomal dominant inheritance.

The patterns followed by single-gene traits within families over a number of generations are determined by the following factors:

1. Whether the trait is dominant or recessive
2. Whether the gene determining the trait is autosomal or X-linked
3. Chance distribution of genes from parents to children through the gametes and
4. Factors affecting the expression of a gene, i.e.,
    a. heterogeneity
    b. pleiotropy
    c. reduced penetrance
    d. variable expressivity
    e. variability of age of onset
    f. sex limitation, complete or partial
    g. interaction of two or more gene pairs
    h. environmental effects

Autosomal Inheritance

Autosomal Dominant Inheritance (Fig. 55)

Examples of autosomal dominant traits are rare conditions like dentinogenesis imperfecta, osteogenesis imperfecta, achondroplasia, porphyria variegata, and common conditions like brachydactyly (short fingers). The overall incidence of autosomal dominants is about 7.0 per 1000.

Usually, in rare conditions, the affected persons are heterozygous, and in common conditions, they may be either homozygous or heterozygous. Usually, the affected person has one affected parent. Very rarely when neither parent is affected, is spontaneous mutation in the gametes the likely cause. The mode of inheritance is as follows:

The father will have genotype T/T, T/t, or t/t depending on whether he is **homozygous affected, heterozygous affected** or **nonaffected.** The same holds good for the genotype of the mother. In each case the gametes produced will be

T/T all T gametes
T/t 50% T, 50% t gametes
t/t all t gametes.

So the possibility of mating types would be as shown in Table 10.

Thus half the children having a heterozygous genotype would be affected, irrespective of the sex of the child.

Autosomal dominant traits show variable expression from minimum involvement to the grossest abnormality. Rarely, the gene may not express itself at all, i.e., it is nonpenetrant resulting in apparently skipped generations in pedigree charts.

TABLE 10: AUTOSOMAL INHERITANCE: MATING TYPES AND EXPECTED PROPORTIONS FOR A PAIR OF AUTOSOMAL ALLELES OF PROGENY T and t e.g., for Tallness in Pea Plant

MATING TYPES		PROGENY	
Genotypes	Phenotypes	Genotypes	Phenotypes
T/T × T/T	tall × tall	All T/T	All tall
T/T × T/t	tall × tall	½ T/T ½ T/t	All tall
T/T × t/t	tall × not tall (dwarf)	All T/t	All tall
T/t × T/t*	tall × tall	¼ T/T ½ T/t ¼ t/t	¾ tall ¼ not tall (dwarf)
T/t × t/t**	tall × not tall (dwarf)	½ T/t ½ t/t	½ tall ½ not tall (dwarf)
t/t × t/t	not tall × not tall (dwarf) (dwarf)	All t/t	All not tall (dwarf)

* This is the usual pattern of inheritance for a rare autosomal recessive trait.

** This is the usual pattern of inheritance for a rare autosomal dominant trait.

TABLE 11: TRAITS OF HOMOZYGOUS AND HETEROZYGOUS INDIVIDUALS

TRAIT	HOMOZYGOUS FOR ONE ALLELE	HETEROZYGOUS	HOMOZYGOUS FOR THE OTHER ALLELE	ALLELIC RELATION
1. Blood groups O, A (dominance involving 2 frequent alleles)	I^AI^A a normal trait	I^OI^A trait as in I^AI^A	I^OA^O another normal trait	I^A dominant I^O recessive
2. Alkaptonuria, Albinism, Phenylketonuria (recessivity of a rare allele)	AA normal	Aa normal	aa abnormal	A dominant a recessive
3. Blood groups M, N (codominance)	I^MI^M a normal trait	I^MI^N a normal trait showing properties of both homozygotes	I^NI^N another normal trait	I^M codominant I^N codominant
4. Catalase in blood (intermediateness)	A^1A^1 normal	A^1A^2 intermediate between two homozygotes	A^2A^2 abnormal	A^1 intermediate A^2 intermediate
5. Polydactyly (dominance of a rare allele)	A^1A^1 normal	A^1A^2 abnormal	A^2A^2 unknown, possibly more abnormal than A^1A^2	A^1 recessive A^2 dominant

Summarizing

1. Among children, whose parents both have a dominant inherited trait i.e., both are heterozygous for the gene responsible for the trait, the genotypic expectations follow Mendel's ratio 1:2:1. One-fourth of the children are homozygous affected and one-fourth homozygous normal.
2. The trait appears in every generation. Normally, there is no skipping of generation.
3. The trait is transmitted by an affected person (heterozygous) to half his children on an average.
4. Unaffected persons do not transmit the trait to their children.
5. The occurrence and transmission of the trait are not influenced by sex. Males and females have equal chances of having the trait and transmitting it.

Autosomal Recessive Inheritance

(Fig.56)

Examples of autosomal recessive traits are cystic fibrosis, albinism, galactosemia, Gaucher's disease, hemoglobinopathies, mucopolysaccharidoses, phenylketonuria, porphyria, Wilson's disease (hepatolenticular degeneration) etc. Most of these conditions are rare. The overall incidence is about 2.5 per thousand.

An autosomal recessive trait is expressed only in the homozygous state, i.e., in an individual who receives the recessive gene from both parents and so is homozygous for it. Males and females have an equal chance of being affected and transmitting the trait. As related individuals are more likely to have the same rare gene than unrelated individuals, consanguineous marriages have a higher probability of producing children affected by a recessive trait. Hence the rarer the recessive trait, the higher is likely to be the proportion of consanguineous marriages.

Usually the affected individual has **heterozygous parents** who are **phenotypically normal.** All the children of the affected person are normal, unless the affected person marries a heterozygote, which (because of the rarity of most recessive traits) is very uncommon. There can be more than one affected person in a sibship.

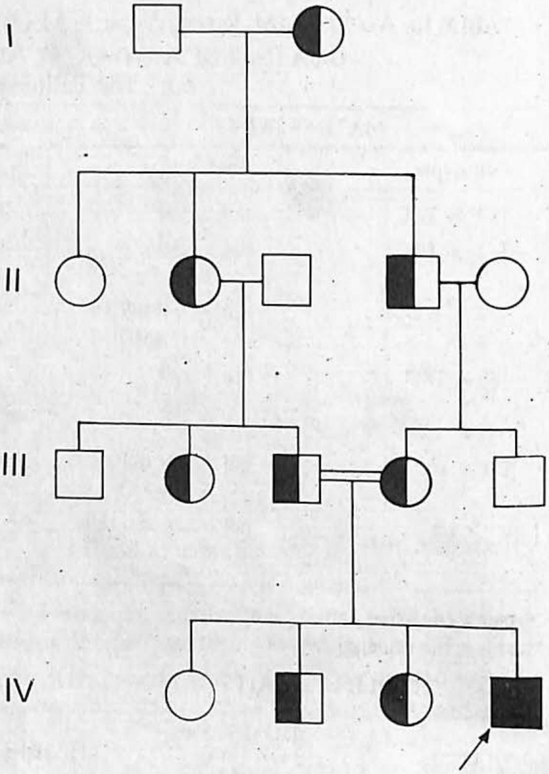

Fig. 56

Typical pedigree chart of autosomal recessive inheritance. Marriage between cousins in the third generation, and its result in the fourth, is shown. The recessive gene was inherited from a common female ancestor (first generation).

The mode of inheritance is as follows:

1. If two affected persons homozygous for the same gene marry, then all their children would be affected; such an event is very rare.
2. If two heterozygotes for the same gene marry, then one-fourth of the children are likely to be affected, one-half would be unaffected heterozygotes, and one-fourth normal.

3. If an affected individual marries a heterozygous carrier, one-half of the children will be affected and the other half will be carriers — unaffected heterozygotes.

Summarizing:

1. An autosomal recessive trait characteristically is seen only in sibs — brothers and sisters. It is not seen inteh parents, offsprings or other relatives.
2. On an average the ratio of affected, carrier and nonaffected is 1:2:1 in sibs; the recurrence risk in such a family is 1 in 4 for each birth.
3. The parents of the affected child may be closely related consanguineously.
4. Males and females are affected equally and transmit the trait equally.

Codominance and Intermediate Inheritance

When both the alleles of a pair are fully expressed in the heterozygote, the genes and the trait are said to be codominant. If a heterozygote is neither like a homozygote with both the dominant genes, nor like a homozygote with both the recessive genes, the genes concerned and the traits are said to show intermediate inheritance.

Examples of codominance are the various blood groups. A person of blood group AB has both A and B antigens on his red cells, showing that allelic genes A and B are fully expressed and therefore codominant.

Sickle-cell anaemia is an example of intermediate inheritance. The homozygote for the abnormal allele has severe sickle-cell anaemia. The heterozygote for the abnormal allele does not have severe sickle-cell anaemia nor is he completely normal. A proportion of his red cells show the sickling phenomenon. Such a heterozygote is intermediate between normal homozygotes and sickle-cell homozygotes; and is said to have the sickle-cell trait. (The usage of the term trait to indicate the clinical features of a heterozygote is not to be confused with usage of the same term in genetics where it means phenotype i.e., obviously visible or detectable expression of a gene). Table 11 indicates the traits of homozygotes and heterozygotes in the various patterns of inheritance discussed above.

Sex-Linked Inheritance

Genes (dominant or recessive) carried on sex chromosomes X and Y, because of the different distribution of sex chromosomes in males and females, produce patterns of inheritance different from genes carried on autosomes. The inheritance of genes on sex chromosomes is known as sex-linked inheritance. For practical purposes sex-linked means X-linked since the only definite Y-linked Mendelizing genes are for hairy pinna and the H-Y antigen. The overall incidence of X-linked disorders is about 0.5 per thousand.

Genes carried on the X chromosomes are referred to as X-linked and genes carried on the Y chromosomes are referred to as Y-linked. Partially sex-linked genes are those genes which are carried on the homologous portions of the X and Y chromosomes.

Examples of X-linked traits are haemophilia, partial colour blindness, vitamin-D resistant rickets, etc while hairy pinna represents a Y-linked trait. Examples of partial sex-linkage are total colour blindness and some rare skin disorders.

X-linked inheritance. A male has only one representative of any X-linked gene (always derived from the mother) and hence he is said to be **hemizygous** rather than **homozygous** or **heterozygous.** Whether recessive or dominant, an X-linked gene is always expressed in the male. A female can be homozygous or heterozygous.

Since the X chromosomes cannot be transmitted from father to son, a male cannot transmit X-linked traits to his male offspring, i.e., father to son. As a father has only one X chromosome which is always transmitted to his daughters he will always transmit the trait to his daughters in the case of dominant genes or render them carriers in the case of recessive genes.

It may be noted here that though a female has two X chromosomes, only one member of the pair is active; the second one remains condensed and relatively non-functional, and is seen as the **Barr body in interphase cells.** Thus, in females, as in males, there is only one functional X, and in heterozygous females, it is a matter of chance whether her paternal or maternal X is in a functional state in a given cell. In heterozygous females, when we refer to X-linked recessive and dominant inheritance, the gene under consideration is functioning only in about half the body cells.

X-linked recessive inheritance. (Fig. 57, 58, 59) A classic example is haemophilia. Other examples are partial colour blindness (an inability to distinguish between shades of red and green), glucose-6-phosphate dehydrogenase (G6PD) deficiency, and Duchenne muscular dystrophy, and probably, testicular feminization syndrome.

The trait is expressed by all males who inherit the gene. Females are affected only if they are homozygous. Thus X-linked recessive diseases are often restricted to males and are uncommon in females.

Mode of inheritance. In haemophilia, the genotypes are XhY for affected male, XhXH for carrier female, XHXH for normal female and XHY for normal male.

1. When an affected male XhY marries a normal female XHXH, all his daughters would be carriers XhXH and all his sons would be normal. The carrier daughters will transmit the haemophilia trait to half their sons.

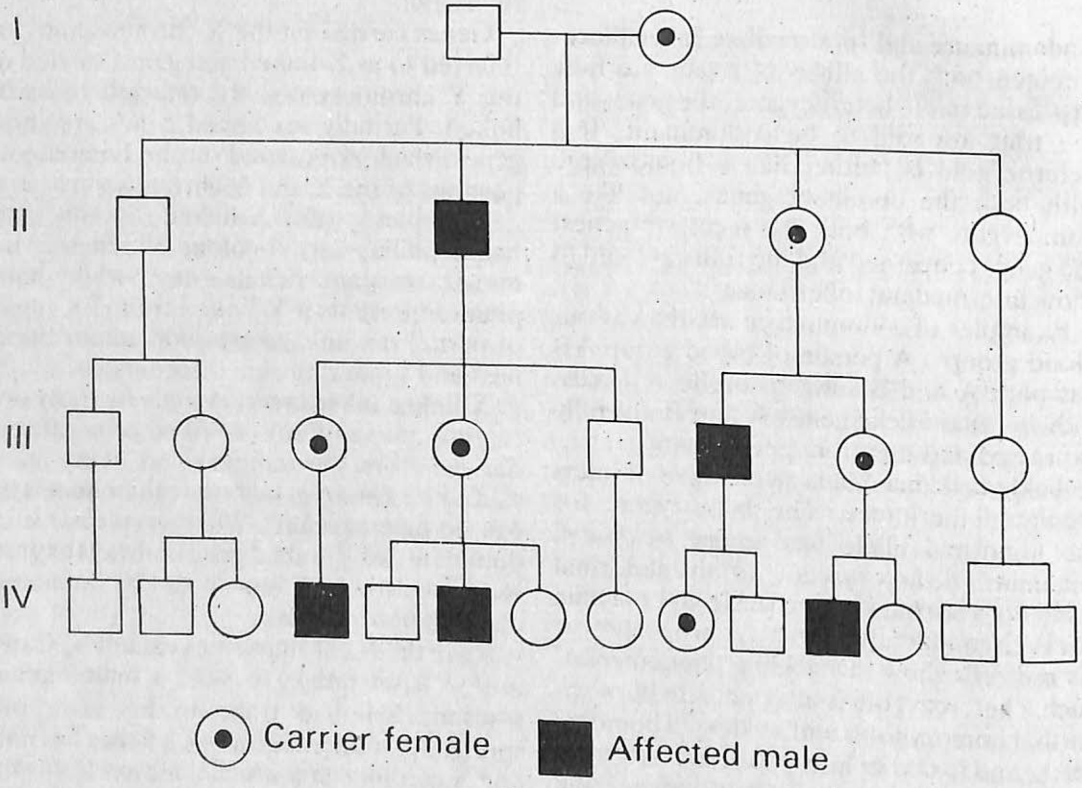

Fig. 57

Typical pedigree of X-linked recessive inheritance in which affected males reproduce, e.g., red-green colour blindness.

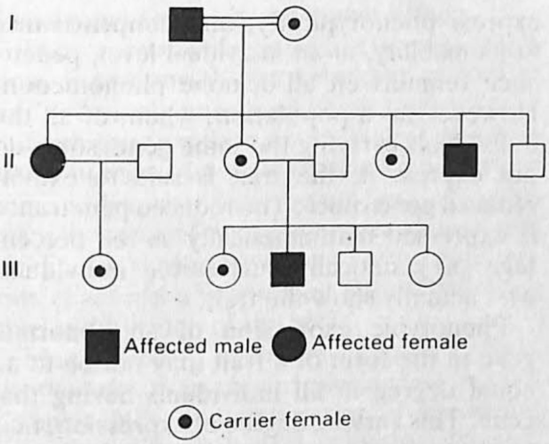

Fig. 58

X-linked recessive inheritance. A pedigree of haemophilia showing between cousins (generation I) and an affected female (generation II).

2. When an affected male XhY marries a carrier female XhXH, half the sons would be affected, half the daughters would be carriers and half the daughters would be affected. However, this type of combination is rare, since haemophilia in females is severe and may be lethal. Female haemophilic offsprings have a good chance of survival with modern therapy.
3. When a carrier female XhXH marries a normal male XHY, half the offsprings would be normal, half the sons would be affected, and half the daugthers would be carrier.

Summarizing:

1. An X-linked trait is mostly seen in males. It is uncommon in females.
2. The trait is transmitted from an affected man through all his daughters to half of his grandsons.
3. The trait is never transmitted from male-to-male, i.e., father to son.
4. The trait can be transmitted through a series of carrier females. The affected males in a family are related to one another through females.

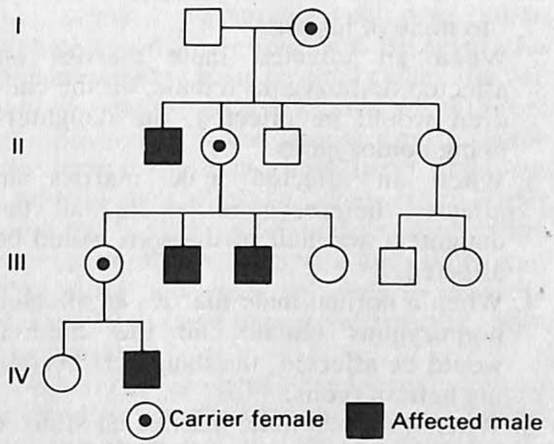

Fig. 59

Typical pedigree of X-linked inheritance, in which affected males do not reproduce, e.g., Duchenne muscular dystrophy.

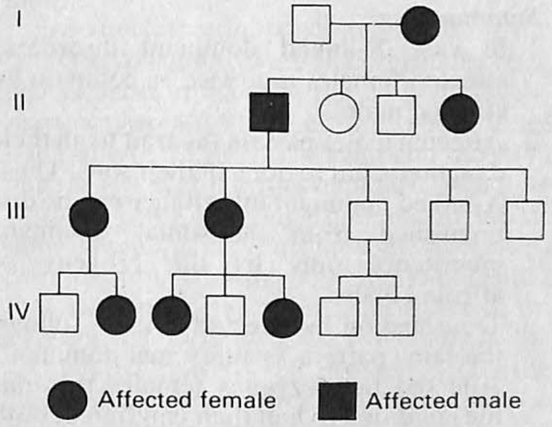

Fig. 60

Typical pedigree of X-linked dominant inheritance.

X-linked dominant inheritance. (Fig. 60). Examples of X-linked dominant traits are vitamin-D-resistant rickets and Xg blood group.

An X-linked dominant trait is seen in homozygous as well as in heterozygous females, and in the male having the gene under consideration on his single X chromosome. As a result, X-linked dominant conditions are twice as common in females as in males. The affected male transmits the trait to all his daughters, but to none of his sons.

Mode of inheritance. 1. When an affected male marries a normal female, he will transmit the trait to all his daughters but to none of his sons.

2. When an affected male marries an affected homozygous female, all the children would be affected, the daughters being homozygous.
3. When an affected male marries an affected heterozygous female, all the daughters and half of the sons would be affected.
4. When a normal male marries an affected homozygous female, all the children would be affected, the daughters becoming heterozygous.
5. When a normal male marries an affected heterozygous female, half the children — sons and daughters — would be heterozygous and affected.

Summarizing:

1. In rare X-linked dominant disorders, affected females are twice as common as affected males.
2. Affected males pass on the trait to all their daughters, but to none of their sons. Thus, X-linked dominant inheritance can be distinguished from autosomal dominant inheritance only by the progeny of affected males.
3. Transmission by affected females follows the same pattern as autosomal dominant. Affected heterozygous females transmit the condition to half their children of both sexes. Affected homozygous females transmit the trait to all their children.

Y-linked inheritance. This is also known as holandric inheritance. An example is hairy ears. As the Y chromosome is only found in males the nonhomologous Y-linked genes are only found in males. Males alone get affected and an affected male transmits the trait to all his sons but to none of his daughters. As the Y chromosome is single, genes on Y chromosome are always expressed phenotypically.

Variation in the Expression of Genes

Penetrance refers to the ability of a gene to express phenotypically, and **nonpenetrance** to its inability, at an individual level, penetrance remains an all-or-none phenomenon. However, in a population, when, of all the individuals carrying the same gene, some do not express it, the trait is said to exhibit **reduced penetrance.** The reduced penetrance is expressed mathematically as the percentage of genetically susceptible individuals who actually show the trait.

Phenotypic expression of an abnormal gene in the form of a trait may not be to an equal degree in all individuals having that gene. This varying degree of expression, i.e., mild, moderate or severe, is called **expressivity.** Thus, in polydactyly, an individual may have only a small wartlike appendage on the side of the hand, but at the other extreme, another affected person may have a complete extra finger. In osteogenesis imperfecta, some individuals are mildly affected so that their everyday life is not disturbed, whereas others are so severely affected that they may become complete invalids with gross skeletal deformities. Most affected individuals, however, lie somewhere between the two extremes.

The phenomena of penetrance and expressivity suggest that expression of genes phenotypically depends at least partly upon modifying genes.

Forme fruste. (literally, frustrated form.) This is any mild clinically insignificant expression of a genetic trait in an abnormality, disease or syndrome. For example, in Marfan's syndrome, an autosomal dominant condition, the full complex of abnormalities manifests as elongated extremities, dislocation of the lens of the eye and cardiovascular abnormalities. In a family affected by this disorder, a generation, through forme fruste may appear to skip the disorder. Here, the ocular and cardiovascular disturbances are so minimal that the individuals are taken clinically as normal. This accounts for skipped generations in autosomal dominant conditions.

Pleiotropy: One Gene—Several Effects

Pleiotropy is a phenomenon where a single gene is responsible for a number of distinct and seemingly unrelated phenotypic effects.

Each gene has the primary role of directing the synthesis of a polypeptide chain. When there is disturbance in the synthesis of such a chain because of the gene defect, there could be diverse secondary effects, if the polypeptide chain has a widespread role in the structure and function of the body.

Many genetic disorders, known as syndromes are examples of pleiotropy. A syndrome refers to a combination of manifestations that commonly occur together. Phenylketonuria is a metabolic disease, inherited as an autosomal recessive, in which the enzyme phenylalanine hydroxylase, essential for the first step in the conversion of phenylalanine to tyrosine, is lacking. This enzyme defect leads to accumulation of phenylalanine in the body, resulting in such diverse effects as mental retardation, excretion of phenylketones in the urine, and hypopigmentation.

Pleiotropy (one gene—several affects) seen in the form of syndromes, is quite different from genetic association and genetic linkage.

Association

It is the occurrence of two or more phenotypic characters in members of a kindred group or a population, in a frequency greater than would be predicted on the basis of chance, i.e., non-random occurrence of two phenotypically separate traits in a population. For example, blood group O and peptic ulcer of the duodenum show significant association. The hereditary basis of peptic ulcer is not established. It is, therefore, very likely that some physiologic peculiarity, and not any genetic linkage with the blood group O, renders a person more susceptible to peptic ulcer.

Linkage

Mendel's law of independent (random) assortment states that during gametogenesis, the members of various gene-pairs assort independently of one another. However, this has been found to be true only when the two genes under study are located on different chromosomes, or are far apart on the same chromosome, when they assort independently of each other, i.e., chances are 50 per cent that both the genes under consideration will pass to the same gamete and 50 per cent that those two genes will pass to different gametes (the same gamete will not carry both the genes).

When the two genes are located close to each other on the same chromosome, there are greater chances that the two genes would be transmitted together to the same gamete. A little note given below explains this exception to Mendel's law of independent assortment.

For the formation of a zygote, both the gametes contribute a set of 23 chromosomes, one member of each of the 23 homologous pairs of chromosomes i.e., the sperm brings haploid 1 to 23 (one of them being X or Y) and the ovum also brings haploid 1 to 23 (one of them being X). Each member of the 23 homologous pairs of chromosomes retains its own identity. Thus the combination of genes that an offspring receives from either of his parents is faithfully carried over in all the subsequent mitotic cell divisions from the stage of zygote onwards throughout all the body cells for the total life span. However, in the gonads where gametogenesis takes place, the meiotic cell division alters the combination of genes on a given chromosome. The two homologous chromosomes, each member derived separately from the two parents, regularly interchange genetic material during the first prophase of meiosis. When the interchanges result in a new combination of genes on homologous chromosomes, the phenomenon is referred to as crossing over or recombination.

The phenomenon of crossing over between the members of a homologous pair i.e., the location of the chiasma is random

This means that the frequency with which crossing over results in the dissociation of the two given loci on a single chromosome will be directly proportional to the distance between them along the chromosome. When two genes are located close to each other on the same chromosome, they tend to be transmitted together to the same gamete much more often. Such loci are termed linked loci.

The term linkage is thus defined as the phenomenon where two genes on a given chromosome are reasonably close together, whereby they do not assort independently of each other but are transmitted to the same gamete more than 50 per cent of the times. The term synteny refers to all genes on the same chromosomes. The syntenic genes are all the genes on any given chromosome, whereas linked genes are very closely placed genes on a given chromosome, where they do not assort independently.

Some of the examples of linkage are genes for the ABO blood groups and nail-patella syndrome, genes for antigen Xg and for partial colour blindness on X chromosome, or genes for Lutheran blood group and for secretor trait (secretion of ABO blood group substances into saliva).

Methods of determining linkage and synteny: Basis of Chromosomal Mapping

1. From pedigree data. When one parent is doubly heterozygous (Aa Bb) and the other parent is homozygous for the recessive allele at each locus (aa bb), this kind of mating is called double backcross. If the loci A and B are not linked, then theoretically

Aa Bb × aa bb
Aa Bb : Aa bb ; aa Bb : aa bb
1 :1 ;1 :1

If this ratio of genotypes is altered, it would mean A and B are linked and thereby not assorting independently of each other. In a single human family with limited progeny, deviations from above mentioned proportions are difficult to note. But observations on a series of large families indicate a significant deviation from 1:1:1:1 ratio which means that A and B are assorting non-randomly i.e., they are linked.

In a person whose genotype is Aa Bb, the two linked genes A and B may be present on the same chromosome; then the genes are said to be linked in coupling and the linkage is in *cis* configuration. (Fig. 61) If the two genes A and B are located one each on two homologous chromosomes, then the genes are said to be linked in repulsion and the linkage is *trans* configuration. To give an example, say, a mother is a carrier for two X-linked genes — a form of colour blindness and haemophilia. If the two genes are linked in coupling, any one son will receive either both the genes or none. If the two genes are linked in repulsion, any one son would receive any one of the conditions. When exceptions occur to this, the cause is recombination between the A and B loci, and the exceptional offsprings are recombinants. The physical basis of recombination is the crossing over of chromosomal material that occurs in meiotic prophase.

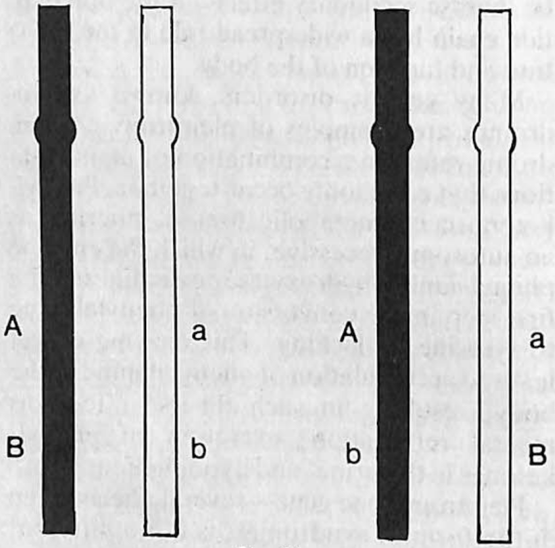

Fig. 61

Linkage may be in coupling AB/ab or in repulsion Ab/aB.

Study of linkage in man is difficult as progeny in humans is small in number and each generation spreads over decades. Here we have to rely on finding informative matings which have occurred by chance. These are rare. For example, in order to determine the relative positions on the X chromosome of the genes for Duchenne muscular dystrophy and haemophilia, we would need to study families where both these diseases occur together. However, these diseases are so rare, that no such family has been described where both diseases occurred together. Hence genetic linkage studies in man are made with the help of **marker genes** in pedigrees of families with a particular hereditary disorder. Marker genes are so called because they are common in the general population with a good chance of their being present in the families under investigation. They show a simple mode of inheritance, have different frequencies in different populations and their phenotypes can be readily classified.

A study of the **autosomal marker genes** has been carried out through the following traits:blood groups, serum protein (haptoglobins, transferrins), ability to taste phenylthiocarbamide (PTC), etc. The X-linked marker traits are colour blindness, Xg blood group, Xm serum factor, glucose-6-phosphate dehydrogenase (G6PD) deficiency, etc.

In human genetics, it is difficult to distinguish between expression of close linkage and expression of a pair of alleles.

Recombination is defined as the occurrence of progeny with combinations of genes other than those that occurred in the parents due to independent assortment or crossing over. If among the offspring 80 per cent have the parental combination of Genes A and B, and 20 per cent have a new combination of Genes A and B not found in either parent, the genes are said to show 20 per cent recombination, i.e., they are 20 map units apart. Distances between any two genes are measured in terms of **map units,** one map unit also known as a **centimorgan,** being equal to 1 per cent of crossing over. The frequency of recombination gives the frequency of crossing over and thereby the distance, between any two loci on a given chromosome, in map units.

2. *Somatic cell hybridization.* This involves getting cells from two tissue culture lines to unite in vitro and the hybrids to form a new viable tissue culture line which can be separated from the parental lines. This technique can reveal to what chromosome a given gene can be assigned. Stocks of **hybrid clones** covering all of 24 different human chromosomes (22 autosomes, X and Y) are available for **linkage studies.**

3. *Hybridization of a specific type of DNA or RNA to a specific segment of a chromosome.* This technique involves combining of nucleic acid strands from disparate sources. The basic process, referred to as *annealing* involves separation of the DNA strand by heat (denaturation) and then renaturation by slow cooling. Combination occurs only if the nucleic acids being annealed contain similar base sequences. If radioactively labelled RNA is present during the reannealing stage, it may band with DNA that has a base pair sequence complementary to its own. After autoradiography, the radioactive RNA establishes the location of the complementary DNA sequence. This is how the genes for 18S and 28S ribosomal RNA have been found to be located on the short arms of the five pairs of acrocentric chromosomes.

4. *Chromosomal aberration mapping method.* Here advantage is taken of the relation of genes to visible chromosomal abnormalities or variations. **Deletion mapping** is the absence of an expected phenotype together with loss of specific chromosomal region. In **duplication mapping,** three alleles expression or 150 per cent of the normal activity of a given enzyme is noted with trisomy for a chromosome or duplication of a chromosome segment.

Chromosomal Mapping

When data from all the techniques – **pedig-**

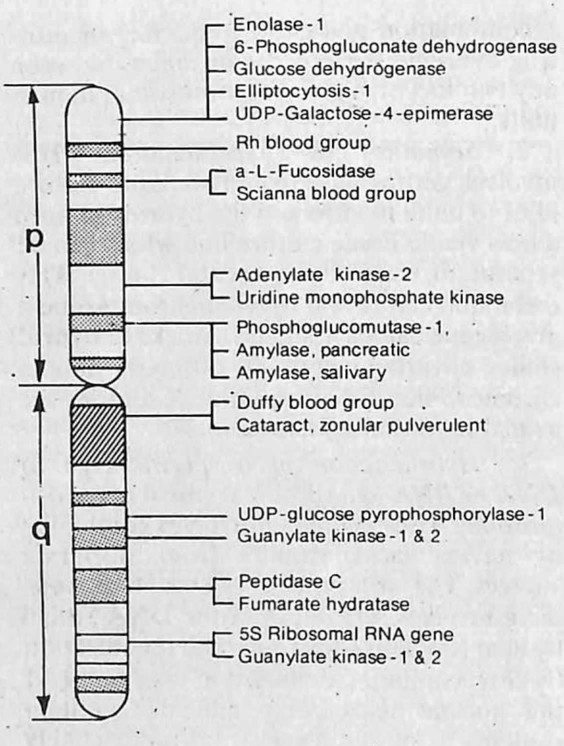

Fig. 62

Tentative map of chromosome-1. Banding pattern also indicated.

ree studies, somatic cell hybridization, chromosomal aberration mapping and nucleic acid hybridization – for linkage studies are synthesised, it has led to the mapping of about 200 human gene-loci. Some of the important ones are the ABO blood group locus on chromosome 9 and the major histocompatibility complex on chromosome 6. Every chromosome has at least one marker gene and chromosome 1 has more than 20.

Tentative maps of the chromosome 1 is given in Fig. 62.

Clinical Application

It is expected that knowledge of chromosomal mapping would be helpful in the detection of heterozygous carriers and of the preclinical states of some genetic disorders, and in antenatal diagnosis.

Genetic Heterogeneity: several genes, each independently having one and same effect

Genetic heterogeneity implies that a particular clinical phenotype can be the result of a number of distinct genetic and enviromental etiologic agents.

Pedigree analyses reveal genetic heterogeneity. For example, in retinitis pigmentosa, in some pedigrees the gene responsible appears to be X-linked, whereas in other pedigrees it appears to be on an autosome. Another example is muscular dystrophies. There are three main types, each differing from the other in the mode of inheritance, age of onset and severity.

If both the parents, with recessively inherited congenital deafness, have children all with normal hearing, it is likely that the parental deafness is caused by different recessive genes, and that each parent has a normal allele at the locus for which the other has an abnormal gene. It is estimated that there are as many as 32 different genes accounting for various forms of inherited deafness.

Sex Limited Traits

A sex limited trait is a trait which is expressed in only one sex though the genes determining it is neither X-linked nor Y-linked.

Since **sexual dimorphism** forms a part of the genetic constitution, it affects the penetrance and expressivity of some genes of the autosomes, which are not themselves concerned with the determination of sex. Some such genes are sex limited in their penetrance, finding expression phenotypically in one sex only. Examples are the anatomical and physiological characteristics of the female sex, such as width of pelvis or age of onset of menstruation. Similarly, sex limited male characters, such as type of beard growth or amount and distribution of body hair, probably depend on genes found in both sexes. An example of a sex limited dominant trait is precocious puberty in the male. Unlike X-linked recessive inheritance,

TABLE 12: EXAMPLES OF AUTOSOMAL PHENOTYPES WITH UNEQUAL EXPRESSION IN MALES AND FEMALES

Phenotype	Mode of inheritance	Sex difference
Baldness	Autosomal dominant in males, recessive in females	Great excess of males
Congenital adrenal hyperplasia	Autosomal recessive	More often recognized in females
Hemochromatosis	Autosomal dominant	Excess of males; loss of blood during menstruation may protect females
Perthe's disease	Autosomal dominant type	Excess of males
Myopathy limited to females	Autosomal dominant	Limited to females
Precocious puberty	Autosomal dominant	Limited to males

sex limited traits can also be transmitted from male to male.

Sex Controlled or Sex Modified or Sex Influenced Traits (Table 12)

When a genotype is expressed in both sexes but in a varying degree, it signifies sex controlled, sex modified or sex influenced genetic expression. For example, harelip and cleft palate are developmental genetic abnormalities, in which penetrance is incomplete, and expressivity varies from very slight external clefts to very severe clefts of the soft and hard palates. Sex influence can be seen from the manifestation that penetrance is higher in males—60 per cent of affected individuals are males and a severe type of expression is seen more often in males. Another example is **pattern baldness** in males.

Variability in manifestation of genetic traits:

1. In age of onset
2. In qualitative expression due to interaction of nonallelic genes.

1. **Age of onset.** This varies from early embryonic life to late age depending on the genetic trait. For example, some lethal chromosomal aberrations are responsible for early abortions. Some conditions are seen as congenital abnormalities (polydactyly). Some genetic diseases are manifested as the child grows—Tay Sachs disease at four to six months, Duchenne muscular dystrophy when the child begins to walk, and acute intermittent porphyria in the young adult. A condition like Huntington's chorea manifests much later in life.

The distinction between a **congenital anomaly** and a **genetic disease** should be clear from this account. Genetic disease means that genes are the causative factors, whereas congenital anomaly means that the disorder is present at birth. In other words, genetic diseases are not necessarily congenital and congenital diseases are not necessarily genetic.

Anticipation. This is a phenomenon of apparent earlier onset of a disease in succeeding generations. In many diseases, earlier onset of age is associated with increased severity leading to progessive worsening of a condition in succeeding generations. This was regarded as the normal course for many inherited disorders. However, it is now felt that the phenomenon of anticipation is apparent rather than real, being more due to a bias in the selection of cases.

2. **Interaction of nonallelic genes.** So far it has been stressed that in a single gene trait, only the allelic genes concerned, are responsible for the manifestation of the trait. However, for a number of genes, their expression is dependent on other genes, i.e. genes are expressed differently on different genetic backgrounds.

For example, the ABO secretor trait represents a situation where two gene pairs act together to produce the phenotype. Secretors are those individuals who secrete the antigens A, B and H in the saliva. To be a secretor of antigen A the person should have both the genes *A* and *Se*.

Multiple Alleles

Alleles are said to be multiple when, in a population, more than two different alleles exist at a given locus of a chromosome. In a given individual only two of these alleles occur, one derived from each parent.

Good examples are various blood groups discussed in Chapter 7 Four alleles A_1, A_2, *B* and *O* for ABO blood groups exist in the population. However, in any given individual, any two different alleles out of these or the same allele in duplicate would be present. One individual can transmit only one of these two alleles to his/her children. Blood groups represent **multiple allelism** where all the alleles happen to be normal. There can be multiple alleles where one is normal and others are abnormal (dominant or recessive). Duchenne muscular dystrophy is a disorder which is transmitted by a recessive gene situated on the X chromosome. Of the two other alleles for the same gene, one is a normal gene and the other a recessive allele expressing as a mild type of muscular dystrophy (Becker type).

POLYGENIC INHERITANCE

Some inherited traits instead of being governed by a single gene (i.e. alleles at one locus), are determined by a number of genes, each having minor effect in expression of a single trait. Such traits are known as **polygenic traits** and the pattern of inheritance is called polygenic.

Some geneticists use the term **multifactorial** as synonymous with polygenic. Others use the term multifactorial trait, where both genetic and nongenetic or environmental factors are involved in determining the trait and where both the genetic and nongenetic factors are multiple.

Polygenic traits are characterised by a **continuous variation,** i.e., they do not manifest themselves as sharply defined pairs of phenotypes like black and white. If a large group of normal human beings were arranged in order of height, each would differ from his neighbours by an exceedingly small measure. The same thing is true for all the traits that can be classified according to a numerical scale like arterial pressure, pulse rate, longevity, degree of resistance to disease, age of onset of disease, IQ, refractive index of eye, dermatoglyphic ridge counts, basal metabolism, or dimension of any particular bodily structure, such as length of finger or weight of lung. Because of this, polygenic traits are also referred to as quantitative traits, and their mode of inheritance as quantitative.

Polygenic quantitative traits show a **Gaussian, bell-shaped** *or* **normal curve** when a specific measurement is plotted on the abscissa against a large number of persons showing that measurement on the ordinate. (Fig. 63). Such a distribution is possible only when the result is determined by a number of genes algebraically additive in their total effect, some acting in one direction, some in the other. If the continuous variation of human stature is largely determined genetically, it must be on the basis of the working together of a large number of genes. Each of these genes would have a small effect, so that the action of no single gene can be individually distinguished. Some of the genes increase the height, some decrease it, thus producing a final result which is the sum total of individual contributions.

The genes that determine ordinary, normal, inherited differences between normal people (differences which are as a rule so clearly quantitative) are the **basic stuff of evolution,** storing a vast reservoir of potential variability, enabling the species to adapt itself to different environments, and also

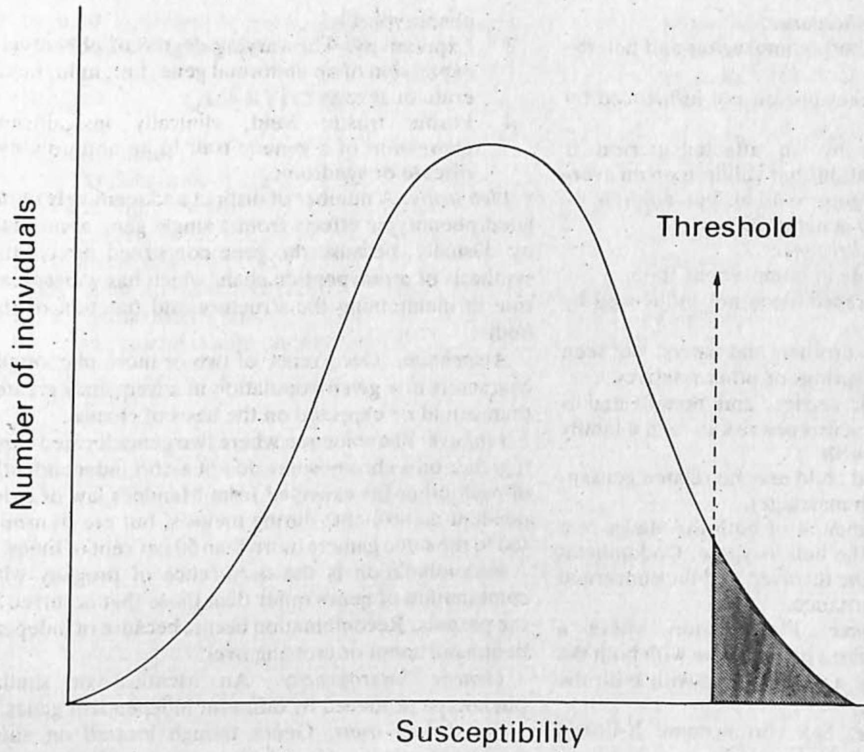

Fig. 63

Susceptibility to a given trait follows the curve of normal distribution but a threshold divides the population into the two phenotypes, normal and abnormal.

enabling it to change gradually and smoothly in response to changing external conditions.

Quasicontinuous Traits

For a polygenic trait having underlying continuous distribution, a threshold effect gives a discontinuous appearance. (Fig. 63). Such a trait is said to be a quasicontinuous or threshold trait.

Quasicontinuous variation is very likely to be the **basis of developmental defects.** If the timing of development shows a continuous variation, extremely slow growth could lead to failure of normal development at a later stage, where punctual completion of this is vital. For example, if the ureteric bud of the mesonephric duct does not complete the development of the collecting tubule system on schedule, the kidney may become polycystic.

The concept of **threshold-controlled liability** to express specific phenotypes (i.e., susceptibility to a given trait is normally distributed, but the population is divided into normal and abnormal type by a threshold) explains the incidence of pyloric stenosis, in the population. The ratio of affected males to affected females is 5:1; the incidence in males is 5 in 1,000, but that in females is only 1 in 1,000. These findings can be accounted for if it is assumed that there are different thresholds for liability to pyloric stenosis for males and females. If in the general population, the threshold for males is lower than that for females, more males than females would be affected.

SUMMARY

Single-gene trait inheritance: Trait determined by genes at a single locus; such inheritance, from one generation to the next, follows Mendel's laws.

Autosomal dominant inheritance

1. Trait expressed in both homozygous and heterozygous states.
2. Occurrence and transmission not influenced by sex.
3. Trait transmitted by an affected person if heterozygous to half his/her children on an average and if homozygous to all his/her children.
4. Trait seen in every generation.

Autosomal recessive inheritance

1. Trait expressed only in homozygous state.
2. Occurrence and transmission not influenced by sex
3. Seen only in sibs – brothers and sisters. Not seen in the parents, offsprings or other relatives.
4. Ratio of affected, carrier, and nonaffected is 1:2:1 in sibs; the recurrence risk in such a family is 1 in 4 for each birth.
5. Parents of affected child may be related consanguineously (cousin marriage).

Codominance: Phenomenon of both the alleles of a pair expressing fully in the heterozygote. Codominant gene and trait are the gene involved and the concerned trait in codominant inheritance.

Intermediate inheritance: Phenomenon where a heterozygote is neither like a homozygote with both the dominant genes nor like a homozygote with both the recessive genes.

Sex-linked inheritance: Sex chromosome X-linked and sex chromosome Y-linked.

X-linked inheritance: 1. No inheritance from father to son. 2. Father always transmits gene to daugher.

X-linked recessive inheritance

1. Always expressed by all males.
2. Females affected only if homozygous.
3. Affected male does not transmit to his sons, transmits to all his daughters.
4. Carrier female transmits to 50% of her children – both male and female.
5. Trait not transmitted from male-to-male.
6. Trait transmitted from affected man through all his daughters to half of all his grandsons.

X-linked dominant inheritance

1. In female, trait manifests in homozygous as well as in heterozygous state.
2. Homozygous female will transmit trait to all the children.
3. Always expressed in male who transmits to all his daughters but to none of his sons.

Y-linked inheritance or holandric inheritance

1. Males only get affected.
2. Affected male transmits the trait to all his sons but to none of his daughters.

Variation in the expressions of genes

1. Penetance: Ability of a gene to express phenotypically.
2. Nonpenetrance: Inability of a gene to express phenotypically.
3. Expressivity: The varying degree of phenotypic expression of an abnormal gene, i.e., mild, moderate or severe.
4. Forme fruste: Mild, clinically insignificant expression of a genetic trait in an abnormality, disease or syndrome.

Pleiotropy: A number of distinct and seemingly unrelated phenotypic effects from a single gene abnormality. Usually, because the gene concerned directs the synthesis of a polypeptide chain which has widespread role in maintaining the structure and function of the body.

Association: Occurrence of two or more phenotypic characters in a given population in a frequency greater than would be expected on the basis of chance.

Linkage: Phenomenon where two genes located close together on a chromosome do not assort independently of each other (as expected from Mendel's law of independent assortment) during meiosis, but are transmitted to the same gamete more than 50 per cent of times.

Recombination is the occurrence of progeny with combination of genes other than those that occurred in the parents. Recombination occurs because of independent assortment or crossing over.

Genetic heterogeneity: An identical or similar phenotype produced by different independent genes.

Sex-lintited traits: Genes though located on autosomes are expressed in only one sex e.g., primary sexual characters, differing in males and females.

Sex-controlled or sex-modified or sex-influenced traits: Genes located on autosomes and expressed in both sexes but to a varying degree in each sex, e.g., secondary sexual characters.

Variability in manifestation of genetic traits

1. Age of onset: Varying from early embryonic life to late age.
2. Interaction of nonallelic genes: For a number of genes, their expression is dependent on other genes.

Multiple alleles: In a population, there may exist more than two alleles at a given locus of a chromosome. In a given individual any two of these alleles occur, one derived from each parent.

Polygenic inheritance: Phenomenon of a trait being governed by a number of genes, each contributing a minor effect in expression of that trait. Traits show a continuous variation i.e., quantitative variation in its manifestation in a population. This can be graphically shown as Gaussian, bell-shaped or normal curve – e.g., human height, pulse rate, blood pressure, blood cholesterol.

Multifactorial inheritance: Multiple genetic and nongenetic factors involved in determining the trait.

Quasicontinuous trait: Polygenic trait continuously distributed dependent on a threshold effect for manifestation. Basis of developmental defects.

QUESTIONS

1. Write notes on:
    Allelomorph or allele
    Multiple alleles
    Linkage
    Pleiotropy
    Quantitative inheritance
2. Give the criteria for:
    Autosomal dominant inheritance
    Autosomal recessive inheritance
    Sex-linked recessive inheritance
    Sex-linked dominant inheritance
    Holandric inheritance
3. Describe factors behind the inheritance of:
    Haemophilia
    G6PD deficiency
4. Write notes on:
    Codominance
    Intermediate inheritance
5. Distinguish between polygenic and multifactorial inheritance.

6 BIOCHEMICAL GENETICS

INTRODUCTION

The chemistry of most living systems centres round the DNA and the wide array of proteins which it codes for. The DNA-protein interactions and interdependence form the basis of such phenomena as cell growth and differentiation, structural and metabolic transformations within cells, and individual variations in responses to drugs. Since all proteins, whether structural or enzymatic are inherited, it follows that any gene mutations are likely to be reflected in the altered structure or in the altered enzyme-dependent metabolic functions.

Biochemical genetics covers the fields of inborn errors of metabolism, the inheritance of normal and abnormal haemoglobins in their embryonic, foetal and adult stages; and pharmacogenetics i.e., the study of genetic variations in responses to various drugs. It also includes the study of factors influencing the inheritance of various blood groups and the immunoglobulins. In view of their outstanding importance in medicine, they have been accorded a separate chapter, which follows.

* * *

Biochemical genetics is the study of genes governing biochemical processes. These are exemplified by inborn errors of metabolism, the haemoglobinopathies, and immunoglobinopathies. Pharmacogenetics is a special area of biochemical genetics that deals with responses to drugs and their genetic modification. Immunoglobinopathies are discussed in Chapter 7.

Inborn Errors of Metabolism

Garrod first put forth the idea, that metabolic processes in living organisms proceed in steps. Each step is governed by one enzyme. **Beadle** and **Tatum** further developed the concept of **one gene-one enzyme,** i.e., each enzyme is the product of one gene. A gene defect leads to a specific total or partial enzyme defect, which in turn leads to a metabolic block, producing pathologic consequences.

An inborn error of metabolism is defined as a genetically determined biochemical disorder in which a specific enzyme defect produces a **metabolic block** that may have pathological consequences. Some characteristic examples are phenylketonuria, alkaptonuria, albinism and galactosaemia. Table 13 gives some examples, their chief clinical manifestations, specific enzyme defect, mode of inheritance, carrier detection and antenatal diagnosis. Such disorders can be classified into those of:

1. **Amino acid metabolism,** e.g., phenylketonuria, alkaptonuria and albinism.
2. **Lipid metabolism,** e.g., Tay-Sachs disease (GM_2 gangliosidosis, infantile amaurotic familial idiocy), Niemann-Pick disease (a sphingomyelin lipidosis)
3. **Mucopolysaccharide metabolism,** e.g., Hurler's syndrome, Hunter's syndrome and other mucopolysaccharidoses.
4. **Purine and pyrimidine metabolism,** e.g., Lesch-Nyhan syndrome.
5. **Copper metabolism,** e.g., Wilson's disease (hepatolenticular degeneration).
6. **Amino acid transport,** e.g., cystinuria.

Most of these disorders are inherited as

TABLE 13: SUMMARY OF GENETICALLY DETERMINED BIOCHEMICAL DISORDERS

Disorder	Chief Clinical Manifestations	Specific Defective Enzyme/Protein	Inheritance	Heterozygote Detection	Prenatal Diagnosis in Cultured Amniotic Fluid Cells
Acatalasia	Oral gangrene; may be entirely normal	Catalase	Autosomal recessive	Low activity of catalase in some but not all types	—
Albinism	Absence of pigmentation in skin and eyes, visual disorders	Tyrosinase (in one common type)	Heterogeneous; usually autosomal recessive, but autosomal dominant and X-linked types exist	—	—
Cystinuria	Aminoaciduria, renal lithiasis	Not known; affects transport of cys, lys, arg, orn.	Type I autosomal recessive; Type II, III incompletely recessive	In Types II and III, increased urinary excretion of cys, lys, arg and orn	—
Galactosaemia	Hepatosplenomegaly, cirrhosis of liver, cataracts, mental retardation	Galactose-I-phosphate uridyl transferase	Autosomal recessive; milder Duarte variant is allelic	Reduced enzyme activity	Absent enzyme activity
Gaucher's disease	Hepatosplenomegaly, neurological problems, anaemia, thrombocytopenia	Glucocerebrosidase	Autosomal recessive; two or more major types	Reduced activity of β-glucosidase in cultured fibroblasts	Absent enzyme activity
Glucose-6-phosphate dehydrogenase variants	Haemolytic anaemia in response to certain foods and drugs	Glucose-6-phosphate dehydrogenase	X-linked; numerous variants	Electrophoretic variants; variants with altered enzyme activity	Theoretically possible
GM_2 gangliosidosis (Tay-Sachs disease)	Degenerative neurological changes, cherry-red spot on macula, severe physical and mental retardation; onset 4 to 6 months of age, death 2 to 4 years	Hexosaminidase A	Autosomal recessive	Reduced level of hexosaminidase A	Absence of hexosaminidase A
Haemoglobinopathies	Anaemia; secondary manifestations depend upon nature of haemoglobin abnormality	Constitutent polypeptide of haemoglobin β or α chain	Autosomal clinical manifestations usually recessive; numerous variants	Usually by electrophoresis	—
Isoniazid inactivation, slow	Neurological problems	Acetyltransferase	Autosomal recessive	—	—

TABLE 13: (CONTINUED)

Disorder	**Chief Clinical Manifestations**	**Specific Defective Enzyme/Protein**	**Inheritance**	**Heterozygote Detection**	**Prenatal Diagnosis in Cultured Amniotic Fluid Cells**
Mucopolysaccharidoses (Hurler's syndrome Type I)	"Gargoyle" facies, mental retardation, hepatosplenomegaly, hearing defect, corneal clouding, cardiovascular problems, dwarfism	Not known; produces mucopolysaccharide accumulation	Autosomal recessive	Excessive storage of certain mucopolysaccharides in cultured fibroblasts	Excessive storage of certain mucopolysaccharides
(Hunter's syndrome Type II)	Similar to Hurler's though corneal clouding is rare	Not known; produces mucopolysaccharide accumulation	X-linked	Excessive storage of certain mucopolysaccharides in cultured fibroblasts	Excessive storage of certain mucopolysaccharides
Niemann-Pick disease	Hepatomegaly, splenomegaly, severe central nervous system damage, cherry-red spot on macula	Sphingomyelinase	Autosomal recessive	Potentially, by assay of sphingomyelinase in cultured fibroblasts	Absence of sphingomyelinase
Pentosuria	Excretion of a reducing substance in urine, but no symptoms of diabetes mellitus	Xylitol dehydrogenase	Autosomal recessive	Intermediate level of xylitol dehydrogenase; abnormal response to loading test	—
Phenylketonuria	Mental retardation, microcephaly, diluted pigmentation	Phenylalanine hydroxylase	Autosomal recessive	Plasma phe and phe/tyr ratio above normal	—
Porphyria, acute intermittent	Episodes of abdominal pain, neurological problems, excessive urinary excretion of δ -amino levulinic acid (ALA)	Excessive production of hepatic ALA synthetase	Autosomal recessive	Patients are usually heterozygous	—
Wilson's disease	Cirrhosis of liver, Kayser-Fleisher ring in cornea, neurological problems	Not known; affects copper metabolism	Autosomal recessive	Decreased serum ceruloplasmin and serum Cu in some but not all heterozygotes	—

autosomal recessives. Some of them are X-linked, e.g, glucose-6-phosphate dehydrogenase (G6PD) variants, hypoxanthine-guanine phosphoribosyl transferase (HGPRT) deficiency (Lesch-Nyhan syndrome), the type of glycogen storage disease associated with phosphorylase kinase deficiency and pseudohypoparathyroidism (an X-linked dominant).

A large number of biochemical defects, which produce inborn errors of metabolism, are now being detected. Usually the mutation in the gene, which codes for the normal enzyme, involves substitution of a single amino acid. Clinically the picture is diverse, ranging in severity from relatively mild, harmless disorders to the lethal.

The clinical picture is the result of disturbances brought about by the **metabolic block** which leads to:

1. **Accumulation of a precursor** just preceding the step where there is a block. The accumulated precursor itself can have toxic effects, or with alternate minor pathways, may lead to production of toxic metabolites.
2. **Stoppage of subsequent steps in the metabolism.** Whenever a feedback mechanism is involved in the control of metabolism, such deficiency would lead to overproduction of the stimulating agent.

Phenylketonuria (Fig. 64) is a typical example. Clinically the child is found to have severe mental retardation; many untreated patients have IQ less than 20. Because of tyrosine deficiency arising from metabolic block, there is reduction in melanin formation. Affected children have blonde hair and blue eyes; regions of the brain which are normally pigmented such as the substantia nigra, may also lack pigment.

In most of these disorders, it is difficult to provide any substitute for the deficient enzyme. The principal treatment is to cut off from the diet those articles of food which are rich in the substances which the patient cannot metabolize, so that these toxic substances will not accumulate. For phenylketonuria, removal of phenylalanine from the diet constitutes an effective treatment. It has been suggested that the **screening of newborn population** should be carried out for conditions like phenylketonuria, since phenylketonuric children are deceptively normal at birth as the maternal enzyme substitutes the missing enzyme during intrauterine development. Treatment is most effective when the diagnosis is made soon after birth and measures instituted immediately. If the child is given phenylalanine even for some time, **irreversible mental retardation** occurs because of accumulation of toxic metabolites of phenylalanine in the brain. The condition can be diagnosed by tests, which detect phenylpyruvic acid in the urine (ferric chloride test) or excess of phenylalanine in the blood **(Guthrie test).**

In a number of genetic disorders resulting from a mutant gene, a particular protein is either not synthesized at all or, although synthesized, is defective. For example in haemophilia there is a deficiency of functionally active antihaemophilic globulin which is required for normal coagulation of blood. In most patients with haemophilia, antihaemophilic globulin is synthesized but is not active, the factor being replaced by a functionally inactive but antigenically similar factor.

Haemoglobins

Haemoglobin is a tetramer with a molecular weight of 64500 Daltons. It consists of 2 α and 2 non-α globin polypeptide chains, each of which has a covalently bound haem group. Each of the four haem groups is made up of an iron atom bound within a protoporphyrin IX ring.

In man, 6 different **globin polypeptide** chains are known. They are called $\alpha,\beta,\gamma,\delta,\epsilon$ and ζ. Each chain consists of a specific sequence of amino acids linked by peptide bonds. The α-chain has 141 amino acids, while the β, γ, δ and the ϵ chains have 146. The ϵ, γ and δ-chains are more like β than like α-chains,

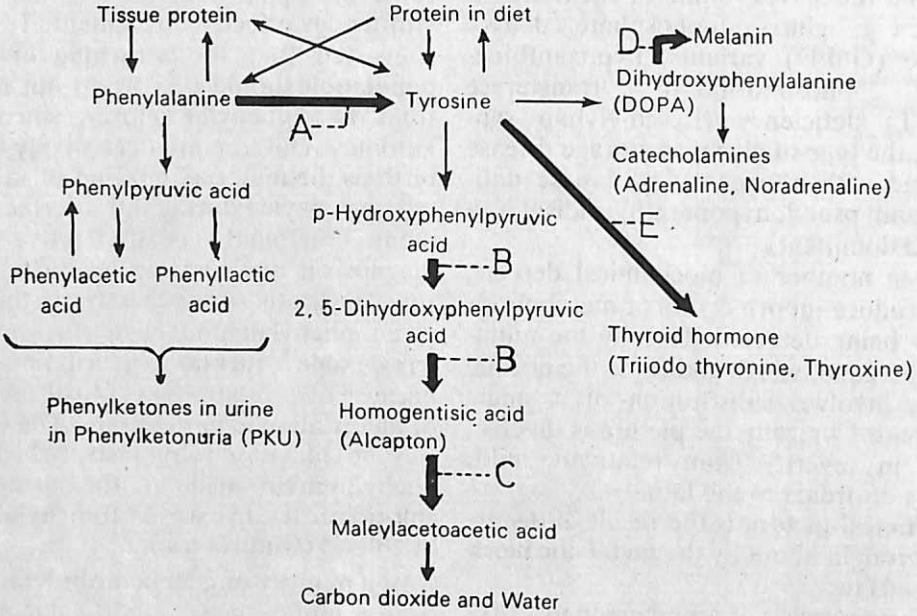

Fig. 64

Scheme showing phenylalanine and tyrosine metabolism. Sites of enzyme block A, B, C, D and E and their consequences indicated.

differing from β at 36, 39 and 10 positions respectively. The two globins ϵ and ζ are found in **embryonic erythrocytes** and while the ζ sequence is incompletely known, it appears to be an analogue of the α-chain.

HbA ($\alpha_2\beta_2$) is usually 92% of the total haemoglobin in normal adults. HbA$_2$ ($\alpha_2\delta_2$) is about 2.5%. HbA$_{1C}$ differs from HbA by the post-translational addition of a glucose at the NH$_2$-terminus of the β chain α_2 (β-N-glucose)$_2$. It is usually about 5%. It is related to the intracellular concentration of glucose and the red cell life span.

HbF ($\alpha_2\gamma_2$) makes up the bulk of haemoglobin (50-85%) in newborns. It declines rapidly after birth, to become 10 to 15% by 4 months of age. (Fig. 65).

Haemoglobins Gower I ($\zeta_2\epsilon_2$), Gower II ($\alpha_2\epsilon_2$) and Portland ($\zeta_2\gamma_2$) are embryonic haemoglobins found in foetuses before 7 to 10 weeks of gestation. At 4 to 5 weeks of intrauterine life, a simultaneous decrease of ζ and ϵ-chain production and an increase in α and γ chain production takes place.

Haemoglobins H and Barts are tetramers of β- and γ-chains respectively. They function very poorly in oxygen transport.

The globin genes are expressed at different times and in different relative amounts during human development.

Genetics of Haemoglobins

In man there are nine different genetic loci which code for the six globin genes. In addition there are at least three pseudogenes, which have sequences similar to other globin genes, but which differ in having some altered sequences which prevent their expression and therefore the production of functional globin chains.

Figure 66 represents schematically the various globin genes and their products.

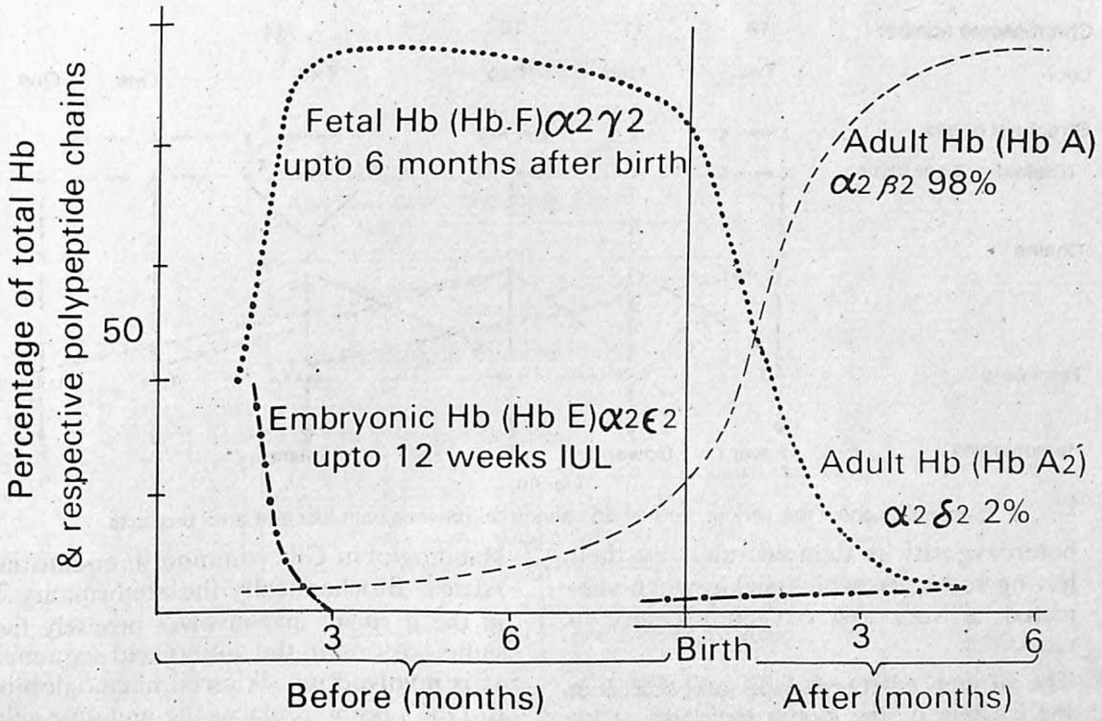

Fig. 65

Normal haemoglobins in prenatal and postnatal life.

Two loci for ζ on chromosome 16. Two loci for α on chromosome 16. One locus for ϵ on chromosome 11. Two loci for γ, i.e., G^{γ} and A^{γ} on chromosome 11. One locus for δ on chromosome 11. One locus for β on chromosome 11.

Since in an individual a diploid set of chromosomes exists, there are 4α and 2β loci in all. The relative numbers of α and β loci are important in understanding the different inheritance patterns of α and β-thalassemias. The β-like genes (ϵ, G^{γ}, A^{γ}, δ) are β located on chromosome 11.

The α-gene complex contains two α loci, two ζ loci and at least one pseudo -α locus ($\psi\alpha$); and is found on chromosome 16.

Haemoglobinopathies

These are a group of disorders characterised by some abnormality of the haemoglobin molecule.

Abnormal haemoglobins

These are produced by mutations in α,β,γ,δ ϵ and ζ genes, i.e., genes which determine the amino acid sequence of the globin portion of the haemoglobin molecule. Several hundred abnormal haemoglobin variants have been detected by now. Table 14 shows the normal and some of the abnormal haemoglobins and their formulae.

1. Sickle-cell haemoglobin (HbS). It was the first abnormal haemoglobin to be detected electrophoretically and is most important clinically. The patient's red cells become grossly abnormal in shape **(sickled)** under low oxygen tension. Fig. 67 shows the important clinical features of sickle-cell disease, which is common in equatorial Africa, the Mediterranean region and in India. Parents of affected children are usually normal clinically but their

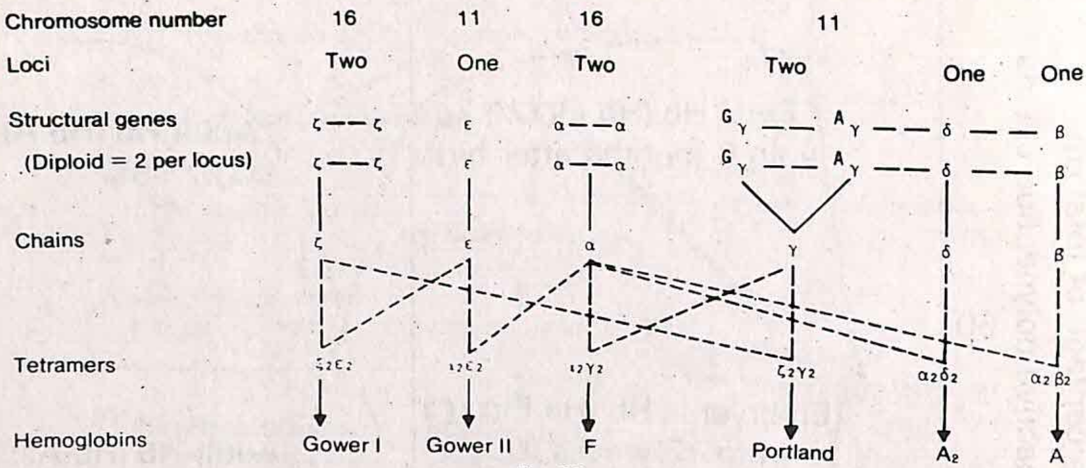

Fig. 66

Scheme to show the various normal and abnormal haemoglobin loci and their products.

heterozygosity is demonstrated by their having red cells, which sickle when subjected to very low oxygen pressure in vitro.

1. The difference between HbA and HbS is in the β chain of the globin molecule. It involves only one of the 146 amino acids in the chain, the amino acid sixth in position from the N-terminal of the β chain. Here, the amino acid valine replaces the glutamic acid of normal haemoglobin, i.e. HbA has a sequence of val-his-leu-thr-pro-*glu*-glu-lys whereas HbS has val-his-leu-thr-pro-*val*-glu-lys. The formula for sickle cell haemoglobin is $\alpha_2\beta_2^s$ or $\alpha_2\beta_2^{s(val)}$.

TABLE 14: EXAMPLES OF NORMAL AND ABNORMAL HAEMOGLOBINS

HAEMOGLOBINS	FORMULAE
Normal	
Hb A	$\alpha_2\beta_2$
Hb A2	$\alpha_2\delta_2$
Hb F	$\alpha_2\gamma_2^{136gly}$ $\alpha_2\gamma_2^{136ala}$
Gower I	$\zeta_2\epsilon_2$
Gower II (Embryonic)	$\alpha_2\epsilon_2$
Variants	
Hb S	$\alpha_2\beta_2{}^s(\alpha_2\beta_2{}^{6glu\rightarrow val})$
Hb C	$\alpha_2\beta_2{}^c(\alpha_2\beta_2{}^{6glu\rightarrow lys})$
Hb Lepore	$\alpha_2\delta\text{-}\beta_2$
Hb Hopkins-2	$\alpha_2\text{Ho-2}\beta_2(\alpha_2{}^{112\ his\rightarrow asp}\beta_2)$
Hb H	β_4

2. Haemoglobin C is common in equatorial Africa. Biochemically the abnormality is in the β chain, and involves precisely the same position in the amino acid sequence as is involved in sickle-cell haemoglobin. In HbC, lysine replaces the glutamic acid of HbA at the sixth amino acid from the N-terminal.
3. Haemoglobin Lepore is an abnormal haemoglobin in which the α chain is normal but the non-α chain has portions of the N-terminal portion of a normal δ, and the C-terminal portion of a normal β chain. The possible mode of origin of Lepore haemoglobin is from unequal crossing over between the two closely linked and very similar genes which code for the β and δ polypeptides. There are 10 differences in amino acid sequence between the δ and β chains. The gene for the anti-Lepore haemoglobin has its first part resembling the β chain and second part resembling the δ.
4. Haemoglobin H is composed of four β-chains (β_4)
5. Haemoglobin Bart is made up of four γ chains (γ_4)
6. Abnormal variant of haemoglobin A2 arises as a result of mutation in the gene coding for the α chains.

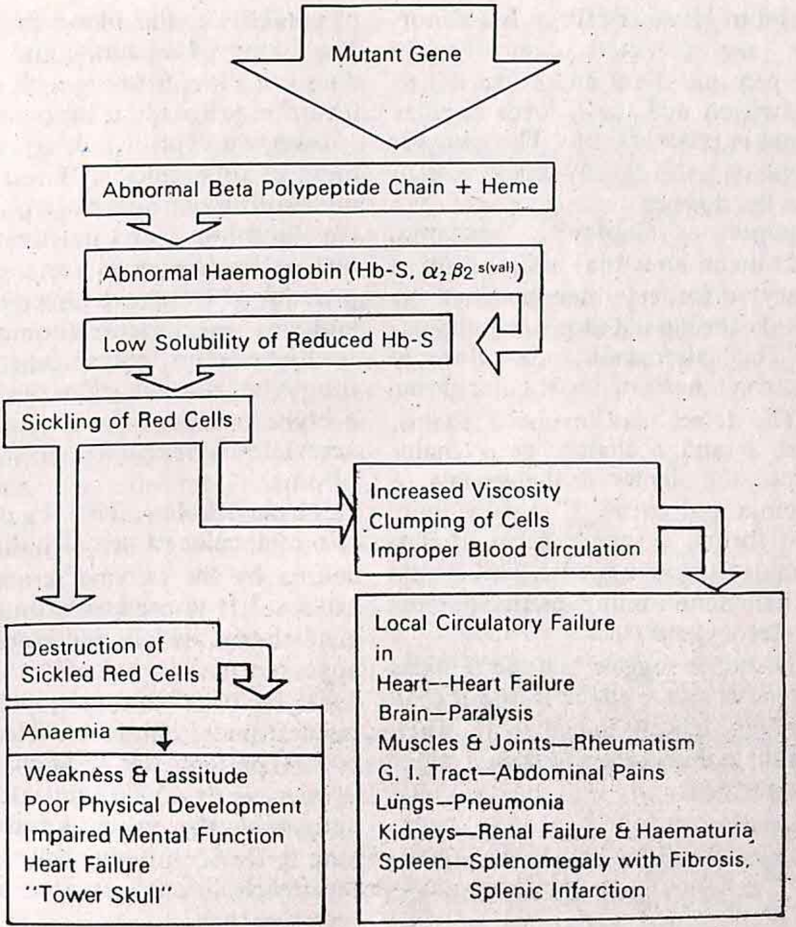
Mutant Gene
Abnormal Beta Polypeptide Chain + Heme
Abnormal Haemoglobin (Hb-S, $\alpha_2\beta_2^{s(val)}$)
Low Solubility of Reduced Hb-S
Sickling of Red Cells
Increased Viscosity
Clumping of Cells
Improper Blood Circulation
Destruction of
Sickled Red Cells
Anaemia
Weakness & Lassitude
Poor Physical Development
Impaired Mental Function
Heart Failure
"Tower Skull"
Local Circulatory Failure
in
Heart—Heart Failure
Brain—Paralysis
Muscles & Joints—Rheumatism
G. I. Tract—Abdominal Pains
Lungs—Pneumonia
Kidneys—Renal Failure & Haematuria
Spleen—Splenomegaly with Fibrosis,
Splenic Infarction

Fig. 67

Scheme to show the formation of sickle-cell haemoglobin and its consequences.

7. Haemoglobin C Georgetown and haemoglobin C Harlem are two abnormal haemoglobins where two separate amino acids are substituted in the same polypeptide chain. Possibly this arises as a result of a second mutation in the β^s allele or intragenic crossing over during meiosis between two β genes carrying mutations at different sites.
8. Haemoglobin Gun Hill ($\alpha_2\beta_2^{93-97}$ deletion) has lost five amino acids in the β chain and haemoglobin Freiburg ($\alpha_2\beta_2^{23}$ deletion) has lost one amino acid in the β chain. These deletions possibly arise as a result of abnormal pairing and intragenic crossing over during meiosis.
9. Haemoglobin Constant Spring has a α chain of 172 amino acids instead of the usual 141, possibly a result of mutation of the codon which terminates the chain, so that the polypeptide continues to add amino acids till the next termination codon halts it.
10. Hereditary persistence of high foetal haemoglobin. In this rare condition, the homozygote has no normal adult

haemoglobin HbA or HbA_2. No abnormalities are detected clinically. In affected persons, the β and δ loci fail to be switched on and the γ locus remains functional in postnatal life. The gene for HbF appears to be closely linked with or allelic to the β gene.

11. Thalassemias (Cooley's anaemia, Mediterranean anaemia) are a group of hereditary disorders characterized by red blood cells having target cell appearance. The abnormality is due to decreased synthesis of a particular globin chain. The defect may involve β chains, δ chains, β and δ chains, or α chains and hence the names β thalassemia, δ thalassemia and so on. Clinically there are two forms, a severe form, thalassemia major (homozygous), and a mild form, thalassemia minor or thalassemia trait (heterozygous)

    Family studies suggest that the β thalassemia gene is either allelic to the β chain gene or very closely linked to it, where perhaps the β thalassemia mutation affects an operator gene.

Pharmacogenetics

Pharmacogenetics is a special branch of biochemical genetics that deals with the genetically determined variations in drug responses.

There is a normal variation in response to all the drugs in a given population. Such a continuous variation is explained on the basis of polygenic inheritance or combination of genetic and environmental factors. But in response to some drugs, there is discontinuous variation, with sharp distinctions between different degrees of response. Usually, discontinuous variation has a genetic basis.

Drugs known to show well-defined genetic variability are hydrogen peroxide, isoniazid, succinylcholine, primaquine, some anticoagulants and anaesthetic agents.

Hydrogen Peroxide. Acatalasemia or acatalasia is a rare recessive trait characterized by absence or greatly reduced activity of catalase in the blood and other tissues. The blood of an individual with acatalasia does not show frothing with H_2O_2; nor does it turn red; instead it turns brownish-black.

Isoniazid. This is a drug used in the treatment of tuberculosis. Based on the rate of metabolism of isoniazid, a population can be classified into rapid inactivators (homozygous or heterozygous for a dominant gene producing izoniazid inactivating enzyme) and slow inactivators (homozygous for the recessive gene). Slow inactivation of this drug is because of lack of the hepatic enzyme acetyltransferase, the enzyme that normally acetylates isoniazid as one step in its metabolism.

Succinylcholine. This is a drug made up of two molecules of acetylcholine and is metabolized by the enzyme serum pseudocholinesterase. It is used as a muscle relaxant in anaesthesia and in electroconvulsive therapy. Normally the effect of the drug—paralysis of all muscles including those of respiration—is transient. However, about 1 in 2000 patients has an **abnormal pseudocholinesterase** or no enzyme at all and in such individuals the paralysis lasts for an unduly long time. Such highly sensitive response to succinylcholine is inherited as an **autosomal recessive trait.**

The activity of cholinesterase in the plasma is determined by two codominant alleles, known as E_1^u and E_1^a. (E_1 signifies the first esterase locus to be described; the superscripts u and a denote genes responsible respectively for the usual and atypical forms of the enzyme). Cholinesterase alteration occurs in persons who are **homozygous for the mutant allele** E_1^a; the enzyme produced by $E_1^aE_1^a$ homozygotes is qualitatively altered and has lower activity than the usual type. The E_1 locus is on chromosome 1. A third allele E_1^s (silent) has been postulated. Either $E_1^aE_1^a$ or $E_1^aE_1^s$ can produce the atypical phenotype, and $E_1^sE_1^s$ produces no activity at all. A fourth allele at the E_1 locus E_1^f, produces a type of cholinesterase in which the enzyme is usually resistant to inhibition by sodium fluoride. A locus on chromosome 16 determining cholinesterase activity, E_2 accounts for a serum cholinesterase isozyme that is detectable only by electrophoresis.

Primaquine. This drug is used for malaria. It is known to be capable of inducing haemolytic anaemia in some patients. The cause of primaquine sensitivity is deficiency in the red cells of the enzyme glucose-6-phosphate dehydrogenase (G6PD). Persons with G6PD deficiency are sensitive to other drugs like phenacetin, nitrofurantoin, some sulphonamides and acetylsalicylic acid. Sensitive individuals also develop haemolytic crisis after eating fava beans (favism). G6PD deficiency is inherited as an X-linked recessive trait.

The specific biochemical role of G6PD is in the hexosemonophosphate shunt pathway, which is the minor pathway for red cell glycolysis. Ninety per cent of red cell glycolysis takes place through the anaerobic pathway. It is only when some drugs are taken that this minor shunt pathway is stressed. When an infection or some other precipitating factor is present, greater number of drugs can cause haemolysis, e.g., acetylsalicylic acid.

Though G6PD deficiency is more common in males, it is possible for females to inherit two abnormal alleles and thus get affected.

Anticoagulants. Coumarin anticoagulants are needed in much higher dosage than normal, in some individuals who have increased resistance to the effects of the drug. The trait is inherited as an autosomal dominant.

Anaesthetic agents. Agents like halothane and succinylcholine bring about a rare complication of anaesthesia—malignant hyperpyrexia—in some individuals. The basic defect in this disorder appears to be a reduced uptake and binding of calcium ions to the sarcoplasmic reticulum. This trait is inherited as an autosomal dominant, with a frequency of 1 in 10,000.

There are some hereditary disorders where the drug response is altered. For example, barbiturates can precipitate an attack of porphyria; sulphonamides can cause severe haemolysis in individuals with haemoglobinopathies such as haemoglobin H, haemoglobin Zurich; chlorthiazide, a diuretic, can precipitate gout in susceptible individuals.

Taste sensitivity to phenylthiocarbamide (PTC) shows genetic polymorphism. Nontasters are homozygous for the recessive allele.

SUMMARY

Biochemical genetics: Study of genes governing biochemical processes – inborn errors of metabolism, haemoglobinopathies, immunoglobinopathies, and pharmacogenetics.

Inborn error of metabolism: Genetically determined biochemical disorder in which a specific enzyme defect produces a metabolic block resulting in abnormal metabolism. Disorder can affect metabolism of amino acids (phenylketonuria), lipids, mucopolysaccharides, purines, pyrimidines, copper, etc. Clinical manifestation results from (a) a metabolic block leading to accumulation of a precursor, (b) stoppage of subsequent metabolic steps leading to deficiency of an essential substance, and (c) overproduction of stimulating agent because of *-ve* feedback. Screening of newborn population may help to intervene very early to prevent gross manifestations of the disease.

Normal haemoglobin: It is a tetramer of 2α and 2 non-α globin polypeptide chains, each having a haem group. Six different globin polypeptide chains exist —α, β, γ, δ, ϵ and ζ. Nine genetic loci code for 6 globin genes, expressed at different times and in different relative amounts during human development. HbA ($\alpha_2\beta_2$) HbA$_2$ ($\alpha_2\delta_2$) are adult haemoglobins, HbF ($\alpha_2\gamma_2$) is foetal haemoglobin. Haemoglobins Gower I ($\zeta_2\epsilon_2$), Gower II ($\alpha_2\epsilon_2$) and Portland ($\zeta_2\gamma_2$) are embryonic haemoglobins.

Haemoglobinopathies: It is a group of disorders characterized by some abnormality of the haemoglobin molecule produced by mutation in α, β, γ, δ, ϵ and ζ genes. Several hundred abnormal haemoglobins are known. Commonly encountered are sickle-cell haemoglobin (HbS), Haemoglobin C, Haemoglobin Lepore. Thalassemias are due to decreased synthesis of any particular globin chain, viz., β, δ, β and δ or α chains.

Pharmacogenetics deals with genetically determined variations in drug response. Individuals with well-defined genetic variability respond abnormally to drugs such as hydrogen peroxide, isoniazid, succinylcholine, primaquine, coumarin (anticoagulant), and anaesthetic agents like halothane. The abnormal response is based on a genetically governed trait which results in quantitative and/or qualitative defect in the enzyme needed for the metabolism of a given drug.

QUESTIONS

1. Write a note on 'Inborn errors of metabolism'
2. Write a note on inheritance of albinism.
3. How are haemoglobins inherited?
4. Write a note on the inheritance of haemoglobinopathies.
5. Write a note on phenylketonuria.
6. Write a note on 'Pharmacogenetics'.

7 IMMUNOGENETICS INCLUDING BLOOD GROUPS

INTRODUCTION

Immunogenetics is that branch of genetics which is concerned with the inheritance of **antigens** and the **antibodies** which react with them in a variety of ways. Antigen-antibody or **immune reactions,** as they are also called, enable the detection of a number of genetic traits. The various blood groups refer to the **surface antigens** carried by the red cell plasma membrane. Their reacting antibodies found in the plasma, may either occur naturally or be induced by antigenic invasion.

The blood groups of paramount clinical importance are the **ABO system** and the **Rh-system.** Apart from their determination in the all-too-frequently resorted to **blood transfusions,** the blood groups have also contributed to an understanding of many **genetic principles** such as **multiple allelism, codominance, genetic polymorphism, immune reactions** and the **study of genetic linkage.**

The immunoglobulins being proteins, represent a class of specifically inherited molecules. They are manufactured by **plasma cells,** which are concerned with a wide range of antigen-antibody reactions involved in **humoral immunity.** Another group of specialized **lymphocytes** is originally produced in the thymus and then gets widely distributed in various other lymphoid organs. These are involved in **cellular immunity** and are mainly concerned in the rejection or elimination of grafted tissue from a source other than one's own body. Since organ transplants are becoming commonplace, a study of different immune-deficiency disorders and their inheritance is of obvious importance.

* * *

Immunogenetics

Immunogenetics deals with the genetic aspects of antigens, antibodies and their interactions. It involves the combination of immunologic and genetic techniques, whereby genetic characters are detected by immune reactions.

Four areas of immunogenetics are of medical significance:

(1) blood groups and clinical problems related to blood group incompatibilities
(2) organ transplantation
(3) immune deficiency diseases and
(4) autoimmune diseases.

Definitions of some of the terms commonly used in immunology given below, help in the understanding of immunogenetics.

Antigen. This is a substance which elicits cellular and/or antibody response by immunocompetent cells and reacts specifically with the cells/antibody so produced.

Antibody. This is an immunoglobulin, a specific complex protein produced in response to the introduction of a specific antigen into an animal and reacts selectively with that antigen.

Immune reaction. This is the specific reaction between an antigen and its specific antibody.

Immunological homeostasis. This is the characteristic condition of a normal adult, who has certain antigens and the ability to react to antigens, by producing cells/antibodies; but who does not produce cells/antibodies to one's own antigens.

Autoimmunity. This is immunity against one's own self. In certain conditions an individual forms antibodies against one or more of his own antigens.

Immunological tolerance. This is the inability to respond to a specific antigen because of previous exposure to that antigen, especially during embryonic life.

Hybrid. This is the progeny of a cross between two genetically different organisms.

Allotypes. This refers to genetically determined differences in antigens.

Incompatibility. The donor and host are said to be incompatible if, because of genetic differences, the host rejects cells transplanted from the donor. In maternal-foetal incompatibility, the mother forms antibodies against foetal cells that enter her circulation.

The **immune response** can be divided into two parts: (1) **humoral, antibody or immunoglobulin,** and (2) **cellular**. In general, antibodies effect the destruction of bacteria, viruses and other microorganisms. The antigen-antibody reaction depends on mutually interlocking sites which vary with the antibody and which are specific for each antigen-antibody pair. An example of cellular immune response is that of lymphoid cells producing killer cells to reject a transplant.

Fig. 68 schematizes the working of the immune system. During the development of an individual, lymphoid precursor cells take one of the two alternative pathways. One path involves formation of the thymus gland, and differentiation into small lymphocytes found in the cortical regions of lymph nodes and spleen. These lymphocytes are responsible for cellular immunity (transplantation immunity, delayed hypersensitivity). The other path involves differentiation of precursor cells into plasma cells responsible for the synthesis of immunoglobulins; and found in Peyer's patches in the intestine.

The basis of the remarkable ability of the body to respond to each of the innumerable antigenic substances by making a specific antibody is not known. It is believed to be due to multiple somatic mutations or to somatic recombinations of genes controlling antibody production. It is suggested that somatic mutations and recombinations occur all the time in that part of the DNA which controls antibody

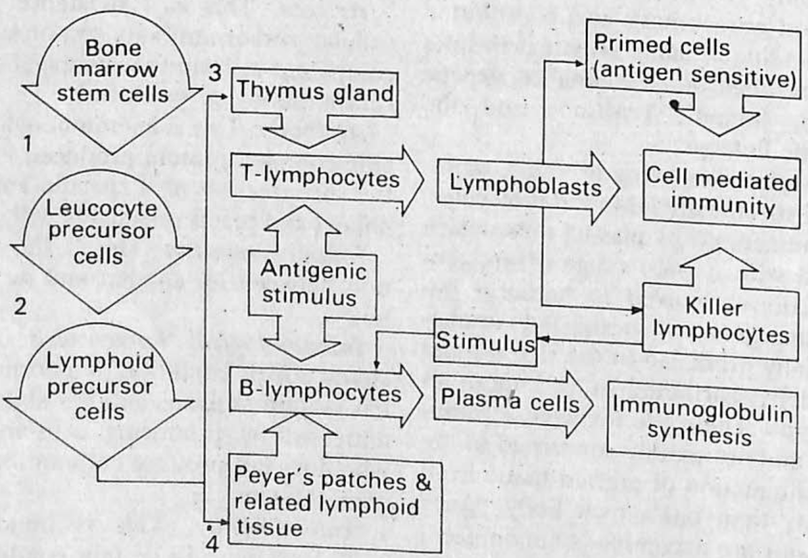

Fig 68
Scheme showing the mode of functioning of the immune system. Block at 1 — reticular dysgenesis; at 2 — Swiss-type agammaglobulinemia at 3 — Di George syndrome; at 4 — Bruton type of agammaglobulinemia

specificity. The outcome is the constant formation of thousands of new antibodies. When an antibody is formed it appears on the surface of the cell of origin. On encountering an antigen which has matching combining sites, the cell carrying the antibody 'locks in' on its antibody production and undergoes repeated divisions. The progeny of that cell now produces only one antibody.

For the cellular immune response, a similar mechanism operates. When a foreign histocompatibility antigen matches an antibody on the surface of a lymphoid cell, the cell commences differentiation and division to produce killer cells that attack the antigen.

Fig. 68 gives sites of hypothetical blocks — 1, 2, 3, 4 — in various immunological deficiency diseases. The different immunological deficiency diseases known so far are discussed on p. 117.

Immunoglobulins

Immunoglobulins are serum proteins which act as antibodies, an important **defence mechanism** against microorganisms. Each immunoglobulin (gamma globulin) molecule is made up of four polypeptide chains: **two identical light** and **two identical heavy chains** held together by **disulfide bonds.** The light chains are of two types, kappa (*K*) and lambda (λ), the two differing in amino acid sequence. The light chains are alike in all classes of immunoglobulins. The heavy chains differ from one immunoglobulin to another.

An immunoglobulin has a special structural-functional organization to suit the varying nature of the antigens and the rather fixed nature of the complement. Any immunoglobulin thus, has a variable region which is concerned with **antigen binding** and a constant region concerned with **complement fixation.** (Fig. 69) The **variable region** along the light chain is designated as V_L and along the heavy chain is designated as V_H. The **constant regions** along the respective chains are designated as C_L C_H The variable regions are specified by the amino acid sequences of the amino terminal portions of both light and heavy chains. The constant region is specified by the carboxy terminal portions of the light and heavy

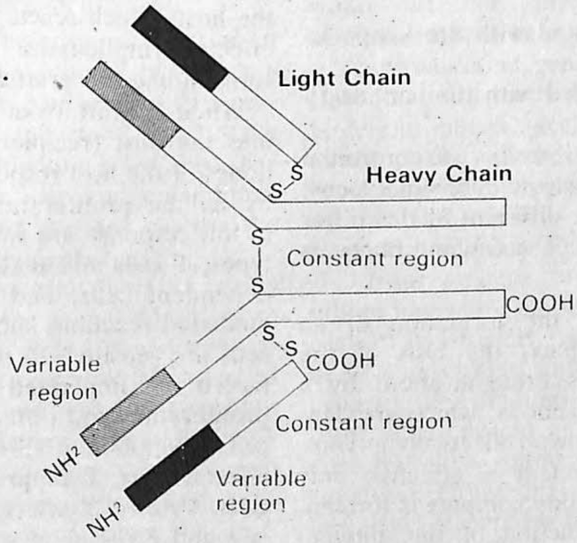

Fig 69
Diagram of an immunoglobulin molecule, consisting of both light and heavy chains with their respective variable and constant regions.

chains. Early in development, each antibody-forming cell begins to produce a single type of heavy chain, selecting these polypeptides from a repertoire of thousands and thousands of possible light and heavy chains.

The regulation of the production of both types of chains involves structural rearrangement of the DNA coding for these polypeptides. Without such DNA rearrangements, the immunoglobulin genes are not expressed. This is discussed in detail on p. 65.

There are five types of immunoglobulins, namely, IgG, IgM, IgA, IgD and IgE. Each has a different H-chain designated respectively as γ, μ, α, δ and ϵ. Both the L-chains are common to all immunoglobulins. The molecular formula of each immunoglobulin is $\gamma_2\ k_2\ \gamma_2\ \lambda_2$ for IgG, $\mu_2\ k_2\ \mu_2\ \lambda_2$ for IgM, $\alpha_2\ k_2\ \alpha_2\ \lambda_2$ for IgA, $\delta_2\ k_2\ \delta_2\ \lambda_2$ for IgD and $\epsilon_2\ k_2\ \epsilon_2\ \lambda_2$ for IgE. All the immunoglobulin types are found in a normal individual.

In a population, the genetically determined variants of the immunoglobulins are:

1. *Gm* system associated with the H-chain of IgG.
2. *Am* system associated with the H-chain of IgA.
3. *Inv* system associated with the kappa L-chain.
4. *Oz* system associated with the lambda L-chain.

The *Gm, Am* and *Inv* groups are controlled by multiple alleles at a single autosomal locus. The frequencies of the different alleles differ in various racial groups. The Gm and Inv traits are polymorphic.

Complement. After the formation of an antigen-antibody complex, the lysis of the antigen-bearing cell is brought about by a sequential series of reactions, which are dependent on proteins known collectively as complement. The complement is effective only after the antigen-antibody complex is formed. It brings about destruction of the antigen-bearing cells by facilitating phagocytosis. The complement is active in the destruction of microorganisms, the production of inflammation and the rejection of implanted tissue.

Hereditary deficiencies of almost all the components of the complement system are known, each associated usually with an autoimmune disease or increased susceptibility to infection. The most common genetic disorder related to the complement is deficiency of the inhibitor of the activated C_1 component. This is the cause of hereditary angioneurotic oedema, an autosomal dominant disorder.

Several components of the complement show polymorphism. So far, all the components that have been mapped are shown to be on chromosome 6, closely linked to the HLA complex.

Transplantation. Tissue and organ transplantation is a modern development. Except for corneal and bone grafts, the greater the antigenic dissimilarity between the donor and the recipient, the greater are the chances of **graft rejection** by the host. Homograft rejection does not take place in identical twins or in those nonidentical twins, where mixing of placental circulation had taken place *in utero*.

The mechanism of rejection is the immune response. The implanted tissue is antigenic to the host, which reacts by the production of T effector lymphocytes. These invade the tissue surrounding the graft and reject the graft.

When a graft from a donor is introduced into the host (recipient), the regional lymph nodes of the host respond to the foreign tissue by cellular proliferation. The important cells in this response are small lymphocytes of two types, **T cells and B Cells.** T cells are thymus dependent cells, and are important in cell-mediated reactions such as tissue rejection. T cells are sensitive to the antigens on the surface of the implanted cells. They respond by proliferation and differentiation to form blast cells (lymphoblasts). **Blast cells** produce **T effector cells, T suppressor cells** and **T helper cells. Primed T effector cells** reach the graft site and bring about rejection. T helper cells are seen as circulating lymphocytes and assist the B cells in response to various antigens. T suppressor cells inhibit the response of B cells to some antigens. Primed cells are antigen-sensitive memory cells, responsible for subsequent rapid response to the same antigen.

B cells are equivalent to cells derived in **birds** from the lymphoid organ called the **bursa of Fabricius** at the distal end of the alimentary canal. B cells produce humoral antibodies. B cells are sensitive to bacterial and viral antigens, and get transformed by antigenic stimulation into plasma cells, which mature by proliferation and differentiation. The plasma cells of one clone synthesize only one antibody.

Types of graft

Autograft. This is a graft made of the host's own tissues.

Allograft. This is a tissue graft from a donor to a host with a different genotype, host and donor being members of the same species.

Isograft. This is a tissue graft between two individuals having identical genotypes, as between identical twins, or between members of an inbred strain of animals.

Xenograft. This is a graft between members of different species, *e.g.*, from ape to man; this type of graft is rejected more rapidly than an allograft.

Allograft reaction. The allograft reaction or primary response is the process by which a host rejects an allograft. The graft at first may appear to be accepted and blood supply to it may be established, but within a few days, the duration depending on the antigenic differences between the host and the donor, the graft dies and is sloughed off.

After rejection of one graft the host rejects a second graft from the same donor more rapidly by a process called secondary-set-response or **hyperacute response.** This type of response is brought about by the primed antigen-sensitive cells (**memory cells**) of the T series. The secondary-set-response is rapid and stronger than the primary response.

Graft-versus-host reaction. When the graft from the donor has **immune-competent cells**, such cells react against the antigens of the host. This is the graft-*versus*-host reaction. The graft-*versus*-host reaction comes in the way of bone marrow transplantation, because the donor tissue containing immune-competent cells reacts against the antigens of the host with serious and often fatal consequences.

Development of immune competence. Sometime before birth, the immune system matures to elicit the immune response. The foetus develops **tolerance** to all the antigens to which its immune system is exposed in the foetal life. Thus the foetus *in utero* not only develops tolerance to its own antigens – **immunological homeostasis** – but is also ready to accept the cells from a genetically different donor to allow them to persist and proliferate, making itself a **chimera** with lifelong tolerance to tissues from that donor. This phenomenon is known as **acquired tolerance**. Some dizygotic twins who happen to exchange *haemopoietic* stem cells *in utero*, have a population of blood cells derived from the co-twin as well as their own. Such a person – **twin chimera** – throughout postnatal life accepts tissue from the co-twin.

Autoimmunity – **autoimmune disease** – is an exception to immunological homeostasis where an individual produces antibodies against antigens of self origin.

Transplantation genetics. The principles of transplantation genetics have been studied largely in inbred mice. Members of an **inbred strain**, some of which are developed by brother-sister mating for well over 100 generations, are virtually identical genetically – **isogenic** – and consequently do not reject tissue from animals of the same strain. An inbred mouse is homozygous at all loci, so that each of its progeny receives one copy of each of its genes. Thus an F_1 (the first generation progeny of a mating) does not regard tissue from either parent as foreign; and therefore does not show immune response of rejection against it. However, if an inbred mouse from one strain is mated with an inbred mouse from another strain, the F_1 hybrid has antigens from the other parent and hence its tissues are not accepted by either parent strain.

In the mouse, there are 25 to 45 **histocompatibility loci (H loci),** each of which has several alleles. The loci are known as H-1, H-2 and so forth; the alleles at a locus are designated $H\text{-}2^{a}$, $H\text{-}2^{b}$ and so on; and the corresponding tissue antigens (H antigens) are $\text{H-}2^{a}$ and $\text{H-}2^{b}$. The major histocompatibility complex (MHC) is the highly complex H-2 locus, which determines powerful antigens that produce a strong T cell response.

In general, in mice, one member of a given inbred strain accepts tissues of another member of the same strain. However, in some mouse strains, females reject tissue from males of the same strain. The antigens responsible for exciting the immune rejection response is determined by a gene located on the Y chromosome. This antigen is known as the H-Y antigen. The H-Y is weak in comparison to the H-2 antigens. The H-Y locus in man and other mammals seems comparable.

The HLA complex. The HLA - **Human Lymphocyte Antigen** – complex is the major histocompatibility complex of man. It is comparable to the H-2 locus of the mouse. The **HLA complex** is a region on chromosome 6 that contains at least four HLA loci as well as several loci related to complement synthesis, the Chido and Rodgers blood group loci and some enzyme loci.

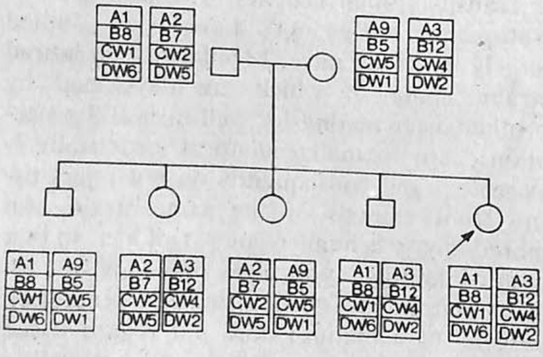

Fig. 70

Pedigree chart of inheritance of HLA haplotypes. A haplotype consists of four loci (HLA-A, HLA-B, HLA-C and HLA-D), each with different antigenic specificities. Since **haplotypes** are transmitted as units, a child has only one haplotype in common with each of his parents, but there is a one-in-four probability that any two sibs will match exactly.

TABLE 15:
PROPORTION OF GENES IN COMMON

Relationship to Proband	Proportion of Genes in Common
Parent, child, sibling	1/2*
Dizygotic twin	1/2
Monozygotic twin	1
Grandparent, grandchild, uncle, aunt, nephew, niece	1/4
First cousin	1/8*
Second cousin	1/32

* The celebrated JBS Haldane once announced that he would lay down his life for two brothers or eight cousins. His reasoning: the survival of two full siblings (each with about half of his genes identical to Haldane's) or the group of cousins (each with about one-eight of his genes the same as Haldane's) made the decision genetically acceptable.

The four HLA loci are designated HLA-A, HLA-B, HLA-C and HLA-D. Each locus has a number of alleles. The set of HLA genes on one chromosome constitutes a haplotype, each with four HLA determinants, for a total of eight HLA antigens. Since haplotypes are transmitted as units, a child has only one haplotype in common with each of his parents, but there is a one-in-four probability that two sibs will match exactly. (Fig. 70). Table 15 gives the antigenic similarity between different members of a family.

Matching tests for donor selection. The histocompatibility, or antigenic similarity between donor and recipient can be determined by matching them for antigens of the ABO blood groups and HLA antigens which are present on various tissues including leucocytes. The matching is done by serological tissue typing and by the mixed lymphocyte culture (MLC) test, in which lymphocytes from donor and recipient are mixed *in vitro*. **Serological tissue typing** is usually carried out by observing the cytotoxic effect of specific antisera on blood lymphocytes collected from the prospective donor and recipient of organs or tissues that are to be transplanted. The MLC test consists in culturing together approximately equal numbers of lymphocytes from two individuals for 5 days. The number of *blast* cells seen in the culture seems to be directly related to the degree of incompatibility between the histocompatibility antigens of the two individuals.

Immune Deficiency Diseases. Most of the immune deficiency diseases are genetically determined. They are the outcome of some form of abnormality in some component of immune response.

The immune deficiency diseases are classified as follows:

1. Swiss-type agammaglobulinemia, where Ig synthesis is depressed. It is an autosomal recessive disease and leads to severe combined immunodeficiency as both B and T cells are absent or greatly reduced.
2. Bruton-type agammaglobulinemia is an X-linked recessive trait. As IgG H-chains and kappa L-chains are synthesized by autosomal genes, it is likely that this disorder is due to a mutation of a particular locus on the X chromosome which normally acts as a regulator gene for the autosomal loci responsible for Ig synthesis.
3. Dysgammaglobulinemias, where only one or two types of immunoglobulin groups are deficient, the remaining being normal or elevated.
4. Hypogammaglobulinemias, where Ig levels are reduced but not totally absent.
5. Ataxia-telangiectasia (autosomal recessive) is characterized by an abnormality of Ig and is associated with a hypoplastic thymus and low levels of IgA.
6. Wiskott-Aldrich syndrome (X-linked recessive) is characterized by an abnormal Ig, and low levels of IgM.

All the immunoglobulinopathies are associated with increased susceptibility to infections and its consequences.

Autoimmune disease. Autoimmunity is the immune response to specific antigens of an individual's own self. Normally such a response does not occur, i.e., a state of immunological homeostasis exists, but this may be disturbed by changes in the antigen-antibody relationship. Exposure to a foreign antigen resembling a self-antigen may result in the formation of a **new antibody** capable of combining with the **self-antigen.** For example, some streptococci on entry into the human body, produce an antigen similar to some of the histocompatibility antigen specific to the cells of the human heart. Hence the antibody formed in response to an infection by those streptococci, apart from attacking the streptococci, also attacks human heart cells. Such an attack may be the causative factor in cardiac damage in rheumatic fever. In rheumatoid arthritis, there is an **autoantibody** produced which combines with normal antibody in plasma. To put it differently, the autoantibody is an anti-antibody in cases of rheumatoid arthritis and is known as the **rheumatoid factor.** Autoimmune diseases affect connective tissue more often. Many of them constitute the so-called collagen diseases.

Based on some observations made on patients of autoimmune disorders and their relatives, it may be tentatively stated that the tendency to form antibodies against self-antigens has a genetic basis.

BLOOD GROUPS

The study of blood groups is important since apart from the boon of blood transfusions, it has contributed to the understanding of many genetic principles such as multiple allelism, codominance, polymorphism, immune reactions and linkage.

The term blood group is restricted, as a rule, to the **red cell antigens.** The notations used for blood groups are mainly of 3 types:

TABLE 16: GENOTYPES, GAMETES AND PHENOTYPES POSSIBLY FORMED FROM THE THREE ALLELIC GENES *A, B* AND *O*

Genotype	**Gametes**	**Phenotype (Blood Group)**
AA	A	A
AO	A or O	A
BB	B	B
BO	B or *O*	B
AB	A or B	AB
OO	O	O

1. Alternative alleles are designated by letter sequence (e.g. *A,B* and *M, N*).
2. Alternative alleles are designated by capital and small letters (e.g. *C* and *c, K* and *k* and *S* and *s*). The small letter does not necessarily signify a recessive trait.
3. Alternative alleles are designated by a symbol with superscript (e.g., Fy^a and Fy^b and Lu^a and Lu^b).

The system followed now is to refer to a blood group after the person in whom the antibody was first recognized (e.g. Duffy and Kidd); and some part of this name is used to designate the gene-locus concerned (e.g., *Fy* for Duffy, *JK* for baby J. Kidd).

ABO Blood Group System

Landsteiner discovered the ABO blood groups—the first blood group system to be discovered. There are **four major phenotypes—AB,A,B and O,** which indicate the presence or absence of corresponding antigen/s on the red cell with O group individuals having neither antigen A nor B. The inheritance of ABO blood group follows the principle of multiple allelism. There are three main alleles designated as I^A, I^B and I^O at the *ABO* locus. The blood group antigen-antibody reaction is described as **isoagglutination,** hence the gene symbol *I*. However, the genes are also referred to as *A,B* and *O,* without the *I*. The antigens A and B of the red cells have naturally occurring antibodies, anti-A (α) and anti-B (β) in the serum, respectively, such that α coexists with group B without interacting and β with group A. α and β occur with group O but are absent in individuals with group AB. This natural occurrence of antibodies is unique to the ABO system. Of the alleles, *A,B* and *O,A* and *B* are codominant while *O* is recessive to both *A* and *B*. (Table 16).

Table 17 shows blood group phenotypes and their relationship with genotypes, serum antibodies, and agglutination reactions within the ABO blood group system.

Table 17 shows that the blood group can be determined by an **isoagglutination reaction,** observed with the naked eye as clumping of red cells. The Table also gives an idea of compatible and incompatible blood transfusions. Though theoretically group O individuals appear as **universal donors** and group AB individuals as **universal recipients,** it is preferable to transfuse a patient only with blood of his own ABO group.

Numerous subtypes of ABO groups have been recognised, e.g., A_1 and A_2, and correspondingly A_1B and A_2B. About 85 per cent of individuals of A group have A_1 subgroup.

The antigenicity of these groups depends upon the three-dimensional arrangement of certain sugar residues added to a series of precursor glycolipids in RBCs, and glycoproteins in body fluids like saliva. These are added by the action of several enzymes known as transferases which are controlled by various genes including *A,B,O* alleles. *Le* (Lewis) gene changes a precursor substance

TABLE 17: BLOOD GROUPS

Phenotype	Genotype	Antigens on Red Cells	Antibodies in serum	Reactions with other blood groups			
				O	A	B	AB
O, α and β	*OO*	neither A nor B	Anti-A (α), Anti-B (β)	−	+	+	+
A, β	*AA* *AO*	*A*	*Anti-B* (β)	−	−	+	+
B, α	*BB* *BO*	B	Anti-A (α)	−	+	−	+
AB, *o*	*AB*	A, B	Neither (o)	−	−	−	−

into Le^a substance. Its recessive allele has no such action and thereby leaves the precursor substance unchanged. The locus H has alleles *H* and *h*. *H* gene transforms Le^a substance into an H substance, the presence of which is essential. For the phenotypic expression of genes A and B The allele O, an amorph, leaves the H substance unchanged

It has been shown that the ABO locus is closely linked with the locus for nail-patella syndrome (autosomal dominant, manifested as dystrophy of nails, absence or reduction of patella with or without other bone dysplasias, and nephropathy) and the locus for adenylate kinase (a polymorphic red cell enzyme which occurs in muscles as myokinase).

Several associations have been suggested between blood groups and susceptibility or resistance to some diseases e.g., Group A and gastric ulcer, group O and duodenal ulcer.

The Bombay Phenotype. The precursor of A and B antigens is H substance or H antigen. *A* and *B* genes act on H substance to modify it to the corresponding antigen, whereas the O gene, an **amorph** or **inactive gene,** keeps H substance unaltered. Most individuals have a gene *H* required for the development of H substance from a precursor. Persons of Bombay phenotype are homozygous for its inactive allele (amorph) *h*. When H is not formed, the enzymes determined by *A* and *B* genes have no substrate on which to act, so that h/h individuals cannot make the A or B antigen even if they have the *A* or *B* gene.

Anti-H antibody is found naturally in serum of subjects of Bombay phenotypes who have genotype *hh*. Because of anti-H antibody, individuals having Bombay phenotypes cannot form A or B antigen even if they carry genotype *AA*/*AO*/*BB*/*BO*. The RBCs of such individuals, inspite of genotypically having *A* or *B* gene, cannot be agglutinated by anti-A, anti-B or anti-H, as their sera contain all three antibodies. This leads to misjudging of blood groups in individuals having Bombay phenotype, which is however very rare. The Bombay phenotype was discovered first in Bombay in 1952.

Secretion of Blood Group Substance

A, B and H antigens of the A, B, O blood group system are also found in other tissues besides red cells. Most individuals also secrete them in a water-soluble form in a number of body fluids. The gene *Se* or **'secretor' gene** determines the ability to secrete; its allele *se* has no known function. **Secretors** (78 per cent of population) of H, A, and B are *Se/Se* or*Se/se* and nonsecretors are *se/se*.

Figure 71 shows the steps involved in the synthesis of H, A and B antigens. The Lewis genes (*Le*) are involved in the synthesis of Le^a and Le^b substances in secretions and plasma.

Table 18 shows the relationship between ABH antigens on the red cells, and the antigens in the saliva of **secretors** and **nonsecretors.**

TABLE 18

Blood group	Secretors		Nonsecretors	
	Red cells antigen	Saliva antigen	Red cells antigen	Saliva antigen
O	H	H	H	—
A	A	A and H	A	—
B	B	B and H	B	—
AB	A & B	A, B and H	A & B	—

The Rh Blood Group System

The Rh blood group system was discovered by Landsteiner and Wiener in 1940 The Rh antigen is named after Rhesus monkeys as they were used in the experiments which led to its discovery. Exact genetic interpretation of the Rh locus is not clear The two systems of nomenclature in current use are those of Fisher and Race, and of Wiener (Table 19).

TABLE 19: THE RH BLOOD GROUP SYSTEM

Fisher and Race Gene Notation	Wiener Gene Notation
CDe	R^1
cDe	R^o
cDE	R^2
CDE	R^z
Cde	r'
cde	r
cdE	r''
CdE	r^y

According to the Fisher and Race postulation, the Rh blood groups are determined by a series of three closely linked genes, *C, D* and *E,* with allelic forms *c, d* and *e*. There are eight different possible combinations of these six genes (*Cde, cde, cDE, cDe, cdE, Cde, CDE, and CdE*). Their frequencies vary in different parts of the world. **No naturally occurring antibodies are known.** Antibodies found in humans after antigenic stimulation, are labelled as anti-LW after Landsteiner and Wiener. Each person has two *Rh* gene complexes on two homologous chromosomes, so that the total number of possible combinations of the eight gene complexes becomes 36. Rh-negative persons are homozygous *d/d* (*C* and *E* are not taken into consideration). However, *Cde, cdE, CdE* are rare as compared to *cde,* so that a large majority of Rh-negative individuals are *cde/cde*. Wiener has postulated a single locus with a large number of alleles. Table 19 gives comparison between Fisher and Race gene notations and Wiener gene notations.

Rh null blood group. Rh null individuals have no Rh antigens. They are comparable to Bombay phenotypes of the ABO blood group system. They do not have a precursor. which is needed as a substrate for formation of the antigens of Rh system. They are homozygous, for a rare gene X^or, whose allele X^1r is responsible for synthesis of a precursor of both Rh and LW antigens (Rh null people also lack LW). The Rh null phenotype is very rare and was first found in an Australian aborigine.

The -D-/-D- Genotype

This is a rare Rh blood type, where, the C and E series of antigens are missing and only D is present. This was attributed to a deletion of that part of chromosome which carries the C and E loci. (It should be noted that the order of the genes on the chromosome is now believed to be *DCE* and not *CDE).* However, the genotype is more likely to be due to a separate suppressor gene like X^or.

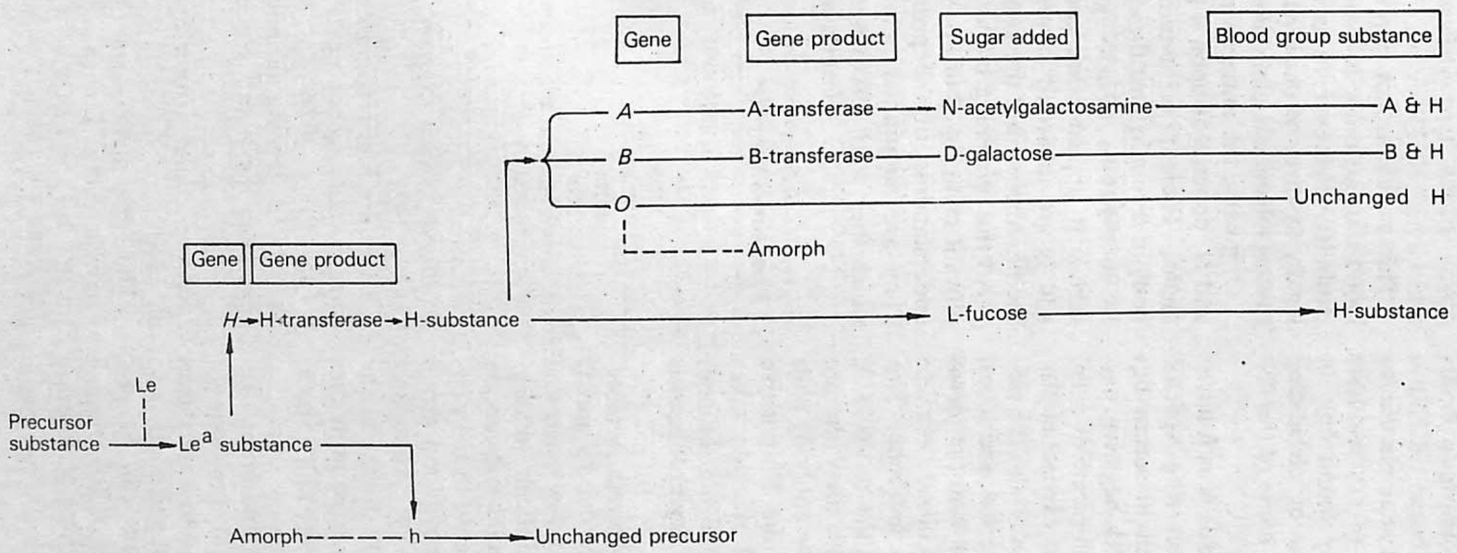

Fig. 71

Schematic representation of the steps involved in the synthesis of H, A and B antigens.

Rh Incompatibility, Erythroblastosis fetalis or haemolytic disease of newborns (HDN) is characterized by jaundice and anaemia due to haemolysis and the presence of erythroblasts in the blood stream normally found only in bone marrow. The presence of **circulating erythroblasts** accounts for the name of the disease.

The **genesis** of erythroblastosis is as follows: There are no naturally occurring specific antibodies against Rh antigen in serum but antibodies are produced in Rh-negative persons by introduction of Rh-positive cells. When an Rh-negative woman carries an Rh-positive foetus, and a break occurs in the placental barrier separating maternal and foetal blood streams, the Rh antigen from the foetus finds its way into the maternal blood stream to excite the production of an antibody. This antibody does not react with the red cells of the Rh-negative mother, since they do not carry the antigen. However, the antibody finds its way through placenta to the Rh-positive foetus, where immunological reaction takes place between the antibody and the red cells of the foetus, culminating in erythroblastosis foetalis.

Most cases of clinically recognized haemolytic disease of the newborn are due to anti-D (anti-Rh) antibody. However a few occur due to anti-A, which are often difficult to diagnose. Such cases tend to be mild and require no treatment. Haemolytic disease in the newborn is rarely caused by other blood group antibodies like anti-K or anti-Fy^a. Antibodies like Anti-Le^a and anti-Le^b are found in the serum of pregnant women but these antibodies are IgM globulins and do not cross the placental barrier.

Prophylaxis against Rh immunization. Rh-sensitization usually occurs at the time of delivery or abortion. Clinical trials have shown that primary immunization of the mother to the Rh antigen on foetal cells can be prevented by giving an injection of Rh-immune globulin to the mother at the time of abortion or delivery.

MNSs Blood Group System (Landsteiner and Levine, 1927)

This system is not important clinically—in blood transfusion or in maternal-foetal incompatibility. However, it is important medicolegally in cases of **disputed paternity** or identity establishment elaborated on p. 137.

Originally the antigens recognized were M and N, dependent upon a pair of codominant alleles. There is no naturally occurring antibody in serum against these antigens. Later on Ss subdivisions of MN groups were discovered. It appears that combinations of MN and Ss are inherited as units. Table 20 gives the phenotype and the genotype.

All the remaining blood groups are not significant clinically, but are studied for a wider understanding of the principles of genetics. They are mentioned below in the order in which they were discovered.

TABLE 20

Blood Group Phenotype	Genotype
MS	M/M/S/S
Ms	M/M/s/s
MNS	M/N/S/S
MNs	M/N/s/s
MNSs	M/N/S/s
NS	N/N/S/S
Ns	N/N/s/s
MSs	M/M/S/s
NSs	N/N/S/s

P Blood Group System (Landsteiner and Levine, 1927)

There are four recognizable phenotypes and corresponding genotypes $P_1 = p^1/p^2, p^1/p^1, p^1/p$; $P_2 = p^2/p^2, p^2/p$; $P = p/p$; and $pk = p_1/p_2$ lacking an independent dominant gene as in the Bombay phenotype.

Lutheran Blood Group System (Callender *et al.* 1946)

The phenotypes and the corresponding genotypes are Lu (a+ b–) = Lu^a/Lu^a; Lu (a+ b+) = Lu^a/Lu^b; Lu (a– b+) = Lu^b/Lu^b, and Lu (a– b–) = silent *Lu* gene or a a dominant suppressor gene affecting the expression of all genes at *Lu* locus. The Lutheran blood group system was the first example studied of autosomal linkage and autosomal crossing over in man — the Lutheran secretor linkage. The locus for myotonic dystrophy is also linked to the Lutheran and secretor loci.

Kell Blood Group System (Coombs *et al.* 1946)

Originally the Kell blood group system was described as having two alternative phenotypes: Kell positive (K+) and Kell negative (k−) determined by a pair of alleles *K* and *k*. Several new antibodies have since been discovered. The Kell system is sometimes found to be responsible for haemolytic disease of the newborn.

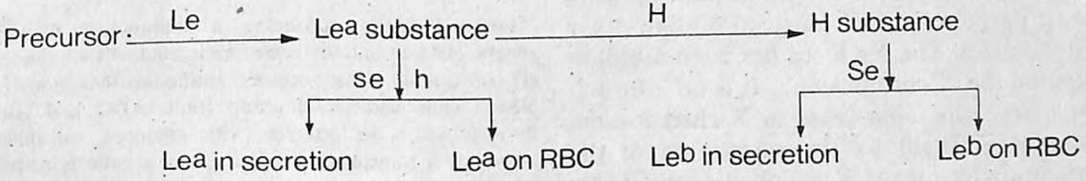

Fig. 72
Schematic representation of the steps involved in the synthesis of the Lewis blood group antigens and its relationship to the ABO blood group system and *secretor* locus.

Lewis Blood Group System (Mourant, 1946)

The common phenotypes and corresponding genotypes are Le (a+ b–) = Le (a+) or Le (a– b+) = Le (b+). Le (a– b–) is rare and Le (a+ b+) is not observed in adults.

Lewis blood group system interacts with the ABO and secretor systems. It is likely that the Lewis substances are not primarily red cell antigens but antigens of the body fluids such as plasma and saliva and that the antigens only get adsorbed on to RBCs from the plasma. (Fig. 72) The presence of Le^a and Le^b antigens in the serum and saliva is determined by a pair of allelic genes, *Le* and *le*. Persons whose red cells are Le (a+ b–) do not secrete ABH substances in saliva, and persons whose red cells are Le (a– b+) always secrete ABH substances.

Duffy Blood Group System (Cutbush *et al.* 1950)

There are three allelic genes at the Duffy locus, Fy^a, Fy^b and Fy^o. The Duffy locus was the first locus to be assigned to a particular chromosome, chromosome No. 1.

Kidd Blood Group System (Allen *et al.* 1951)

There are three allelic genes at Kidd locus, Jk^a, Jk^b and Jk. Anti-Jk^a is responsible for haemolytic disease of the newborn and for some transfusion reactions.

Diego Blood Group System (Layrisse *et al.* 1955)

Di^a antigen is suggested to be a specific marker for Mongolians. The phenotypes are Di *(a+)* and Di *(a–)* for the genotypes Di^a/Di^a or Di^a/Di, and *Di/Di* respectively.

I Blood Group System (Wiener *et al.* 1956)

This system is different from the other blood group systems. Almost all adults are I positive. In fact I antigen was believed to be a public antigen possessed by all members of the human species. The foetal antigen i is strong at birth and decreases in strength thereafter, whereas the adult antigen I is poorly developed at birth and increases as i decreases. Very rarely are adults I negative.

In many blood disorders in the adults, like thalassemia major, the red cells are agglutinated by both anti-i and anti-I.

Yt (Cartwright) Blood Group System (Eaton *et al.* 1956)

The phenotypes and corresponding genotypes are Yt (a+ b–) = Yt^a/Yt^a; Yt (a+ b+) = Yt^a/Yt^b; and Yt (a– b+) = Yt^b/Yt^b. About 98% of the population are Yt (a+).

Xg Blood Group System (Mann *et al.* 1962)

This blood group system is of special interest to geneticists. As the Xg locus is on X chromosome, it is used as an X-linked marker.

The Xg locus on the X chromosome appears not to participate in X inactivation like other X-linked loci. The Xg locus has been useful in mapping the X chromosome. It is an informative marker in some cases of X chromosome aneuploidy. It allows the calculation of the population frequency of X-linked genes. Table 21 gives the phenotypes and genotypes.

TABLE 21: PHENOTYPES AND GENOTYPES

Sex	Phenotype	Genotype
Male	Xg (a+)	Xg^a/Y
	Xg(a–)	Xg/Y
Female	Xg(a+)	Xg^a/Xg^a
	Xg(a–)	Xg/Xg

Dombrock Blood Group System (Swanson *et al.* 1965)

The known phenotypes and corresponding genotypes so far are Do (a+ b–) = Do^a/Do^a; Do (a– b+) = Do^b/Do^b. Approximately 64 per cent of northern Europeans are Do (a+).

Blood group chimeras

A person with blood cells of two different genotypes derived from two different zygotes is called a blood group chimera. In blood group chimeras, the **exchange of haemopoietic cells** takes place in foetal life, prior to the development of immune competence.

The foreign cells do not stimulate the host to form antibodies against them, so they persist in the host's bone marrow and remain throughout life. If only haemopoietic cells are exchanged and not the germ cells, the host will transmit to his offspring only his own blood group.

The possible mode of origin of blood group chimeras is sharing of their circulations in prenatal life by dizygotic twins or dispermic chimeras, formed by fusion of dizygotic twin zygotes or early embryos. Such possibilities are rare and hence incidence of blood group chimeras is very low.

SUMMARY

Immunogenetics is looking at immunology through genetic glasses, and *vice versa*. An individual's immunologic constitution is his antigens, antibodies, lymphocytes, plasma cells and blood group (both ABO and Rh), through which he interacts with microbes, transfused blood, or a transplanted organ. Some genetic principles have been derived from the study of such interactions.

Antibodies or immunoglobulins need a system of complements for successful functioning. Hereditary deficiencies of various elements of the complement system lead to a form of autoimmune disease (destructive immune action against one's own self), and increased susceptibility to infections. All components of the complement system mapped so far are on chromosome 6, closely linked to the HLA complex.

Transplantation genetics—part of immunogenetics—is studied largely in inbred mice. A mouse has 24 to 45 histocompatibility loci or H loci, each with several alleles, and known as H-1, H-2, and so on. The H-2 locus is the Major Histocompatibility Complex (MHC) determining antigens that evoke a strong T cell response in a donor. The Human Lymphocyte Antigen or HLA complex is the MHC in man, comparable to the H-2 locus in mice. The HLA complex comprises 4 loci (HLA-A, HLA-B, HLA-C, HLA-D) and is located on chromosome 6. The greater the similarity between the HLA complex of donor and of recipient, as also between their blood groups, the greater the chances of a successful transplantation.

Immune deficiency diseases—agammaglobulinemias, dysgammaglobulinemias, hypogammaglobulinemias, and so on—are immunoglobulinopathies that lead to increased susceptibility to infections. Autoimmune disease arises

when antibodies and immune-competent lymphocytes attack an individual's own tissues. Rheumatoid arthritis, rheumatic myocarditis, and collagen disorders are probably autoimmune in nature. Autoimmunity is currently held responsible for the ageing process.

Blood groups. Knowledge of blood groups accounts for what is the most successful and frequent tissue transplant the world over — *blood transfusion*. Study of blood groups has also contributed to the understanding of many genetic principles such as multiple allelism, codominance, polymorphism, immune reactions and linkage. It also facilitates organ transplantation.

The term blood group refers to red cell antigens. Major blood group systems are ABO and Rh. Generally, group O persons are universal donors and AB individuals are universal recipients. However, a patient should preferably be transfused with the blood group belonging to his own group. Blood group antigens are also found in other tissues, besides red cells. Most individuals secrete them in body fluids, notably in saliva and these are called secretors. Those not secreting are called non-secretors. Rh incompatibility underlies *erythroblastosis foetalis* or haemolytic disease of newborn. Rh *-ve* mother reacts against her Rh *+ve* foetus leading to haemolysis, anaemia, jaundice, etc. Therapy consists in appropriate transfusion of blood to the newborn.

QUESTIONS

1. Describe the genetics of the ABO blood group system.
2. Describe the genetics of the Rh blood group system.
3. What are multiple alleles? Explain with examples.
4. What is polymorphism? Explain it with examples.
5. Write a note on the genetics of organ transplantation.
6. What are immunoglobulins? Write a note on their molecular structure. Mention their significance.
7. Write a note on the HLA system.

8 POPULATION GENETICS

INTRODUCTION

A population is to a human geneticist what *E. coli, Drosophila* or mice are to an experimental geneticist. Since human generation time is of the order of twenty-five years, human genetics is not amenable to direct laboratory observations. However, an entire human population manifesting an infinite range of **genetic variability** of different traits is available for instant observation, through the application of **mathematical** and **statistical techniques.**

Known phenotypes can lead us to knowing the related genotypes, from which the frequencies of different alleles in a given population can be readily ascertained on the basis of the **Hardy-Weinberg law or principle.** The law states that in a randomly mating population, if the frequency of a pair of alleles determining a particular trait is represented as p and q such that $p + q = 1$, then the three genotypes would be expected to occur in the frequencies of p^2, 2pq and q^2. Further these frequencies will tend to remain constant from generation to generation in a very **large inbreeding population,** provided factors like natural selection, mutation, *etc.,* do not disturb this equilibrium.

Population genetics has sometimes been called **bean-bag genetics,** since if identical black and white beans are mixed in a known proportion in a bag and drawn out two at a time by random chance without looking, the frequencies with which both whites, one black and one white and both blacks are likely to occur, would be as mentioned for p^2, 2pq, and q^2.

Twins are a special category of a human population. They may be identical or fraternal. Genetic studies can help to ascertain their **zygosity,** *i.e.,* whether they are monozygous or dizygous. Monozygotic twins, in particular, are a rich source of genetic material, whereupon different environmental effects can be studied.

Population studies have recently been extended to a detailed analysis of **dermal ridge patterns** on the palms, fingers, thumbs, the soles of the feet and the toes. These are known to be inherited in a polygenic manner and have shown characteristic variations in a number of chromosomal abnormalities.

* * *

POPULATION GENETICS

Population genetics is the study of genes in populations. It deals with the distribution and behaviour of genes and with how gene and genotype frequencies are maintained or changed. The change in the gene and genotype frequencies is the basis of evolution. Hence the study of population genetics also incorporates the study of factors concerned in human evolution.

Gene Frequencies in Populations

The Hardy-Weinberg principle is the basis of population genetics. It was put forward independently by an English mathematician, **G. H. Hardy,** and a German physician, **W. Weinberg,** in 1908.

The Hardy-Weinberg law states that if two alleles (A and a) occur in a randomly mating population with the frequency of p and q, respectively, where $p + q = 1$, then the expected proportions of the three genotypes would be $AA = p^2$, $Aa = 2pq$, and $aa = q^2$

(Table 22). The proportion remains constant from generation to generation in an infinitely large, inbreeding population. **Mutation, selection, migration** and **genetic drift** can disturb the **Hardy-Weinberg equilibrium.**

If the two genes A and a are in proportion to p and q, the sperms and ova will contain them in the same proportions, and with random mating, the various gametic combinations, would be:

Male Female
A(p) a(q) × A(p) a(q)
AA (p^2) + Aa(pq) + Aa(pq) + aa(q^2)

Therefore, the frequencies of the various offspring from such matings would be p^2 (AA), 2pq(Aa) and q^2(aa).

TABLE 22: RELATIONSHIP OF GENE FREQUENCY TO GENOTYPE FREQUENCY

Gene Frequencies		**Genotype Frequencies**		
		p^2	$2pq$	q^2
p	q	*A/A*	*A/a*	*a/a*
0.5	*0.5*	*0.25*	*0.50*	*0.25*
0.6	*0.4*	*0.36*	*0.48*	*0.16*
0.7	*0.3*	*0.49*	*0.42*	*0.09*
0.8	*0.2*	*0.64*	*0.32*	*0.04*
0.9	*0.1*	*0.81*	*0.18*	*0.01*
0.99	*0.01*	*0.98*	*0.02*	*0.0001*
0.999	*0.001*	*0.998*	*0.002*	*0.000001*

If the progeny were to mate among themselves, the result would be

AA(p^2) Aa(2pq) aa(q^2) × AA(p^2) Aa(2pq) aa(q^2)

AA/AA $= p^2\times p^2 = p^4$
AA/Aa $= p^2\times 2pq = 2p^3q$
AA/aa $= p^2\times p^2 = p^2q^2$
Aa/AA $= 2pq\times p^2 = 2p^3q$
Aa/Aa $= 2pq\times 2pq = 4p^2q^2$
Aa/aa $= 2pq\times q^2 = 2pq^3$
aa/AA $= q^2\times p^2 = p^2q^2$
aa/Aa $= q^2\times 2pq = 2pq^3$
aa/aa $= q^2\times q^2 = q^4$

$$\begin{aligned}\text{i.e., Total} &= p^4+4p^3q+6p^2q^2+4pq^3+q^4\\ &= p^4+2p^3q+p^2q^2+2p^3q+4p^2q^2\\ &\quad +2pq^3+q^2p^2+2pq^3+q^4\\ &= p^2(p^2+2pq+q^2)+2pq(p^2+2pq\\ &\quad +q^2)+q^2(p^2+2pq+q^2)\\ &= p^2(p+q)^2+2pq(p+q)^2+q^2(p+q)^2\\ &= p^2+2pq+q^2, \text{ since } p+q=1\end{aligned}$$

This shows that the proportions of the various genotypes remain the same in the second generation as they were in the first. If one continues to calculate for many generations, the result would be the same. The relative proportions of the genotypes would remain constant from one generation to another, and would occur in the proportions $p^2 : 2pq : q^2$.

Based on this equation it is possible to find out the frequency of the various genotypes in the population if the frequency of one of the homozygotes is known. If the frequency of an autosomal trait is 1/40,000

$q^2 = 1/40{,}000$
$q = 1/200$
and, $p = 1—1/200=199/200$.
The frequency of carriers = 2pq

$$= 2\times\frac{199}{200}\times\frac{1}{200}$$
$$\cong 1/100$$

This also illustrates the fact that in the general population carriers are more frequent than affected individuals.

Factors affecting Hardy-Weinberg equilibrium:

The Hardy-Weinberg equilibrium is altered if there is non-random mating. **Random mating (panmixis)** ensures that the frequencies of the different kinds of matings are determined only by the relative frequency of the genotypes in the population. In practice, the requirement of random matings is not commonly fulfilled. The preferential selection of a mate with a particular genotype is common and such a mating is referred to as **assortative or nonrandom mating.** Preference for a mate on the same genotype is positive assortative mating and preference for a mate of a different genotype is **negative assortative mating.** Consanguineous mating is a special form of assortative mating. It disturbs the Hardy-

Weinberg equilibrium by reducing the heterozygotes and thus increasing the proportion of homozygotes.

Mutation is another factor disturbing the Hardy-Weinberg equilibrium. For details on mutation, see Chapter 4.

Natural selection is an important factor operative in evolution. The **Darwinian theory of biological fitness** is considered to be the relative ability of an organism to survive and transmit its genes to the next generation. It is determined by the number of offspring who reach reproductive age. Fitness is unity (or 100 per cent) if a person has at least two such offsprings. In the modern era, survival of the fittest is interpreted as operative through the action of selection upon new genotypes which have arisen by mutation or recombination. Autosomal dominant genes are always expressed and are exposed to the scrutiny of selection, in contrast to autosomal recessive genes. As a result, the effects of selection are more obvious and can be more readily measured for dominant genes than for recessive ones.

Genetic drift is the fluctuation of gene frequencies in small populations due to new settlements formed by members of an older group. **Gene flow** is a gradual diffusion of genes from one population to another by **migration** and **miscegenation.**

Twins

Man and other large mammals, normally bear only one offspring at a time. Multiple births result from the simultaneous intrauterine development of two (or more) embryos. The commonest form of multiple births is twinning — the birth of a pair of foetuses. Multiplicity above two becomes rarer as the number of foetuses increases. The overall incidence of twinning is 1 : 80-90 births, and that of identical twinning is 1 : 270 births.

Twins provide important study material in genetics. Galton in 1875 had drawn attention to the importance of twin studies for comparison of the effects of heredity and environment, or nature and nurture. Twins are said to be **concordant** for a given trait if both exhibit the trait. For diseases presumably caused by genetic factors, **monozygotic twins** show a higher concordance rate than **dizygotic twins.** If monozygotic twins do not show full concordance for a given condition, it can be concluded that nongenetic factors are also playing a part in its etiology.

Classification of twins (Fig. 73)

1. Like, true, identical, uniovular, or monozygotic
2. Unlike, false, nonidentical, fraternal, binovular or dizygotic
3. Conjoined twins, i.e., double monsters and Siamese twins

1. **Identical or homozygous** twins are derived from a single fertilized ovum, whereby each member of the pair acquires an identical chromosomal constitution. Developing from a single blastocyst, they are usually contained within a common chorionic sac and share a common placenta. The umbilical cord and the amniotic cavity also may not be separate. The twins have identical physical features, the same sex and the same blood group. Monozygotic twinning is a hereditary character.

Uniovular but **dispermatic twinning** has also been reported. Here, either the mature ovum, the secondary oocyte, or the primary oocyte undergoes an equal cytoplasmic division instead of throwing off a small polar body. Each of these three variants has different degrees of genetic dissimilarity depending upon the stage at which such a division occurs.

Dispermy represents a rare condition which is a converse of twinning. It may provide a triploid or a tetraploid constitution to the zygote depending upon whether two sperms fertilize a single haploid or diploid ovum, or a haploid ovum which has fused with its polar body. Thus in the same individual, two genetically dissimilar sets of cells could occur.

2. **False twins** are about three times more common than true twins, but this ratio may be reversed, as for example in the Japanese population. They are due to the simultaneous fertilization of two ova from the same or different

ovarian follicles by separate sperms. Each has its own chromosomal constitution, chorionic sac, and placenta. The sex and blood group may or may not be the same. The physical features show resemblance, but are not identical. Dizygotic twinning is also partly a hereditary trait.

3. **Conjoined twins** are a variety of true twins who are joined with each other to a small or large extent. Such "double monsters", are known as **Siamese twins.** They arise from the incomplete separation of the two embryos developed from a single zygote.

Determination of twin zygosity. The determination of zygosity — whether monozygous or dizygous — of a twin pair helps to conduct research in genetic and developmental disorders in twins. It also helps in the selection of donors in cases of transplantation of tissues and organs. In transplantation, a monozygotic co-twin is the most useful donor, whereas a dizygotic co-twin is genetically like any other sibling.

Zygosity is determined by the examination of the placenta and foetal membranes, and by using various genetic markers indicating the similarities and differences between the co-twins.

Chimerism. A chimera is an individual whose cells have arisen from more than one fertilized egg, as in the following examples:

1. Conjoined twins.
2. Anastomosis of placental blood vessels in twin pregnancies, resulting in the mixing of blood and mixed cell-lines.
3. In a leukaemic patient, repopulation of bone marrow from a donor, following suppression of the host marrow by irradiation.
4. Rarely, somatic cells throughout the body may indicate their chimeric origin. If the two types of cells are XX and XY, the individual's sexual differentiation may be abnormal.

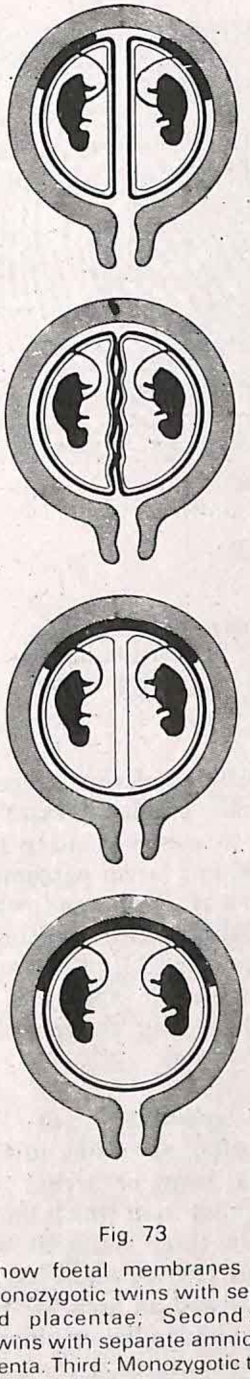

Fig. 73

Scheme to show foetal membranes in twins. First: Dizygotic or monozygotic twins with separate amnions, chorions and placentae; Second: Dizygotic or monozygotic twins with separate amnions and chorions but single placenta. Third: Monozygotic twins with single chorion and placenta but separate amnions. Fourth: Monozygotic twins with single amnion, chorion and placenta.

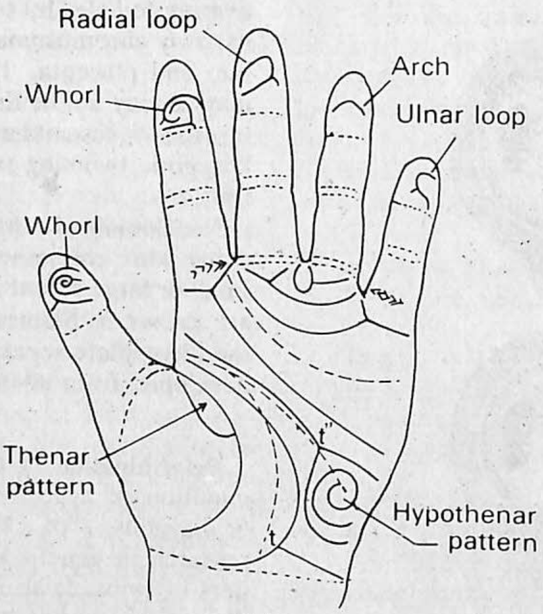

Fig 74

The nomenclature of the dermatoglyphics of the palm and fingers. There are four digital triradii (a, b, c and d) and an axial triradius t (t'' indicates its distal location, t''' indicates location further distal to t''). Thenar, hypothenar and digital patterns are shown.

Dermatoglyphics

Dermatoglyphics is the study of patterns of the ridged skin of the palms, fingers, soles and toes. The importance of dermatoglyphics in genetics is because:

1. Characteristic combinations of pattern types are found in Down's syndrome and other chromosomal disorders.
2. Dermatoglyphic traits are genetically determined.
3. They provide a useful means of measuring resemblance between twins in the determination of twin zygosity.

The patterns studied are:

1. The flexion creases of the palm.
2. Dermal patterns (Fig. 74).
   a. Fingerprints
   b. Palmar patterns
   c. Plantar patterns.

1. **The flexion creases:** The 'heart', 'head' and 'life' lines of palmistry are formed at the same time as the dermal ridges. They also affect the course of dermal ridges. A single transverse crease (**simian crease**) in place of the usual two creases is found in 1 per cent of Caucasians and in a larger percentage of Asiatics. In Down's syndrome and other chromosomal abnormalities, single flexion creases are very common (Fig. 33). Simian creases are also more common in abnormal individuals, such as children with congenital malformations.

2. **Dermal patterns:** (a) Fingerprints. They are classified according to Galton's system as **whorls, loops or arches** (Fig. 75). A **triradius** is a point from which three ridge systems course in three different directions at angles of about 120°. A whorl has two triradii, a loop has one and an arch has none. Loops are subclassified as radial or ulnar, depending upon whether they open to the radial or ulnar side of the finger. In a fingerprint formula, the patterns are conventionally listed as:

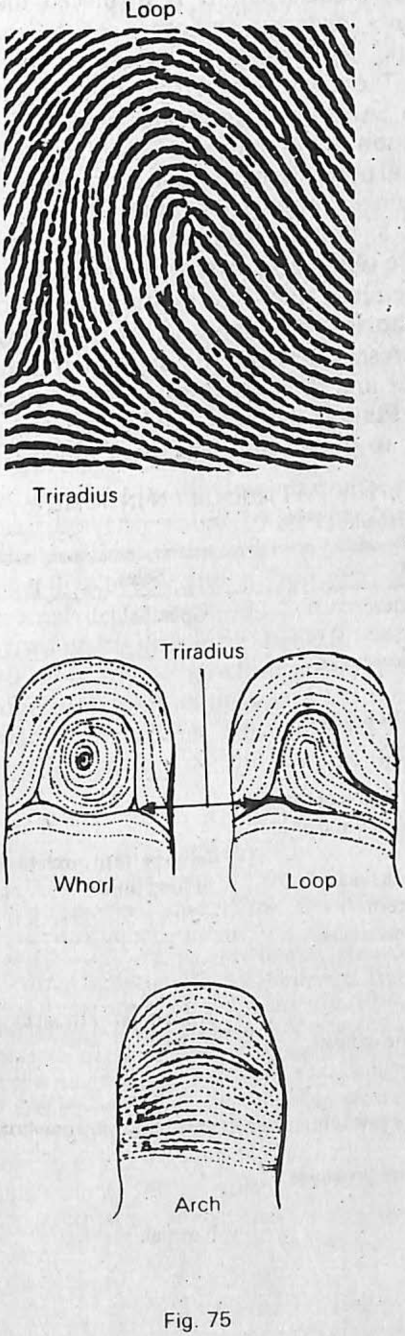

Fig. 75

Diagrams to show different types of fingerprints. The magnified picture of the loop shows rows of sweat pores. For ridge counting, the line drawn from the triradius to the core of the loop is used.

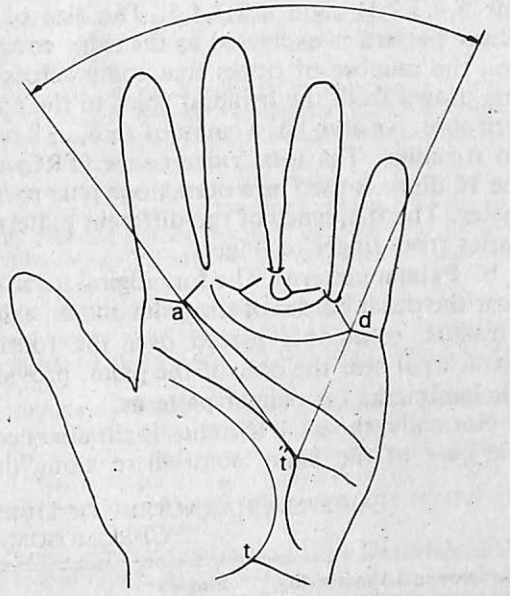

Fig. 76

Diagram to show the measurement of the atd angle of the palm. If there is more than one axial triradius, the distal one is used. Usually the angle measures around 60°. Angles of more than 75° are common in cases of Down's syndrome, where the axial triradius is shifted distally.

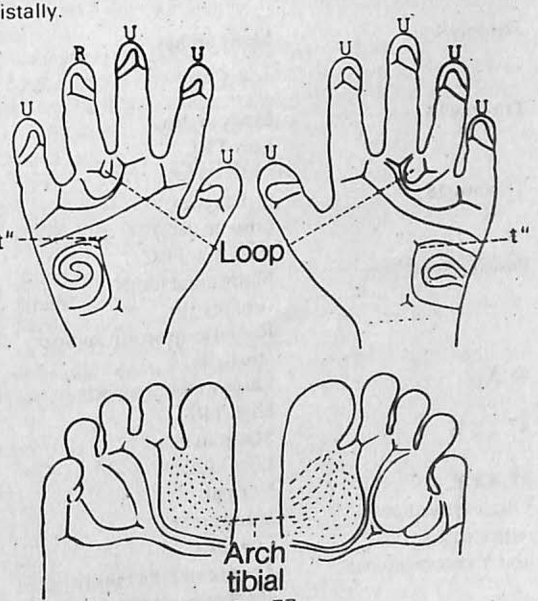

Fig. 77

Typical dermal patterns in Down's syndrome, many ulnar loops, radial loop on 4th digit, axial triradius t" giving atd angle of 80° and tibial arch in the sole.

left 5,4,3,2,1; right 1,2,3,4,5. The size of a finger pattern is expressed as the **ridge count,** i.e., the number of ridges that come across a line drawn from the triradial point to the pattern core. An arch has a count of zero, as it has no triradius. The total ridge count (TRC) of the 10 digits is used as a dermatoglyphic parameter. The frequency of the different patterns varies from finger to finger.

b. **Palmar patterns.** The four digital triradii, near the distal border of the palm and an axial triradius, commonly placed over the fourth metacarpal near the base of the palm, provide the landmarks for palmar patterns.

Normally, the axial triradius is situated near the base of the palm, somewhere along the fourth metacarpal. It is displaced distally in Down's syndrome and other chromosomal disorders. Its location is measured as the "atd angle" or in terms of the total length of the palm. And at angle greater than 57° is more common in patients with Down's syndrome and several other chromosomal syndromes than in the general population. (Fig. 76).

In a normal palm the ridges commonly course obliquely towards the proximal portion of the ulnar side. Interdigital patterns of loops or whorls are formed, if the recurving ridges are present between the digital triradii. Hypothenar and thenar patterns may be present.

c. **Plantar patterns.** These patterns are difficult to obtain and hence not much is known

TABLE 23: CHARACTERISTIC DERMATOGLYPHIC PATTERN SEEN IN SOME CHROMOSOMAL ABNORMALITIES

Chromosomal Abnormality	**Fingers**	**Palms**	**Soles**
5p–	Many arches Many whorls Low TRC	Thenar pattern t" Single flexion crease	Open field
Trisomy 8	—	t' or t" Uni or bilateral single flexion crease	—
Trisomy 9p	Many arches	t" Absence of fusion of the sub-digital triradii b and c	—
Trisomy 13	Many arches Low TRC	t" or t''' Single flexion crease Thenar pattern	large pattern, arch fibular or loop tibial
Trisomy 18	6-10 arches (also on toes) Very low TRC	Single flexion crease	—
Down's Syndrome	Many ulnar loops (usually 10) Radial loop on 4th and/or 5th digits	t" Single flexion crease	Arch tibial or small loop distal
45,X	Large loops or whorls High TRC	t slightly more distal	very large loop or whorl
47,XXY	Many arches Low TRC	t slightly more proximal	—
47,XYY	Normal	Normal	Normal
Other syndromes with extra X and Y chromosomes	Many arches Low TRC The greater the number of sex chromosomes, the lower the TRC		

Abbreviations: t = axial triradius; t" = distal displacement of axial triradius; t''' = extreme displacement; TRC = Total Ridge Count about them. In various clinical syndromes, well-defined hallucal area patterns have been desrcibed. About 50 per cent of Down's syndrome cases show an unusual **"arch tibial"** pattern. (Fig. 77).

Usually, dermatoglyphics show bilateral symmetry. At times asymmetry is seen, and is referred to as **Fluctuating Asymmetry.** This random deviation from bilateral symmetry may have some correlation with congenital abnormality like cleft palate with family history.

Clinical Applications

1. **Chromosomal abnormalities.** The various chromosomal disorders are often associated with some unusual dermatoglyphic patterns. (Table 23). The differences are mainly seen in (a) the Total Ridge Count (TRC) which may be higher or lower (b) the flexion creases of palm, which may be represented by a single crease and (c) the position of the axial triradius which tends to get displaced distally. However, any of the abnormal dermatogylphic features, on its own, carries no significance unless it is associated with other abnormal clinical features.
2. **Congenital malformations.** Congenital malformations involving limbs are associated with gross distortion of the dermatoglyphic patterns.

SUMMARY

Population genetics: Studies the distribution and behaviour of genes in populations.

Hardy-Weinberg Law: Gives gene frequencies in populations, enunciated independently by Hardy and Weinberg. It states that if two alleles (A and a) occur in a randomly mating population with the frequency of p and q respectively, where $p+q=1$, then the expected proportions of the three genotypes would be $AA = p^2$, $Aa = 2pq$, and $aa = q^2$. The proportion remains constant from generation to generation in an infinitely large, inbreeding population.

Factors affecting Hardy-Weinberg Law: Mutation, natural selection, migration and genetic drift.

Twins: Birth of a pair of foetuses simultaneously

Classification:

1. Like, true, identical, uniovular or monozygotic; derived from a single fertilized ovum; share a common placenta and have identical physical features, the same sex and same blood groups.
2. Unlike, false, nonidentical, fraternal, binovular or dizygotic; due to simultaneous fertilization of two ova by separate sperms. Each of the twins has its own chromosomal constitution, chorionic sac, and placenta. They may or may not be of same sex.
3. Conjoined twins: a type of true twins joined to one another to a varying extent, arising by incomplete separation of two embryos developed from a single zygote.

Usefulness of study of twins: 1. Research in genetic and developmental disorders 2. Comparison of effect of heredity and environment in causation of diseases 3. Transplantation — selection of donors.

Chimera: An individual whose cells have arisen from more than one fertilized egg.

Dermatoglyphics: Study of patterns of the ridged skin of the palms, fingers, soles and toes.

Palm: Flexion creases, palmar patterns—four digital triradii and an axial triradius.

Fingers: Fingerprints — whorls, loops, arches; triradius — point from which three ridge systems course in three different directions at angles of 120°; finger pattern expressed as ridge count, i.e., the number of ridges that come across a line drawn from the triradial point to the pattern core.

Sole: Planter pattern—studied less often.

Usefulness of dermatogylphics: Study of chromosomal disorders and congential malformations. Both are associated with some unusual dermatoglyphic patterns.

QUESTIONS

1. State and explain the "Hardy-Weinberg principle" or law.
2. State the factors which disturb the Hardy-Weinberg equilibrium.
3. Write a note on the genetic importance of the study of twins. How are twins classified?
4. What is dermatoglyphics? State its importance in genetic studies.

9 GENETICS AND THE DOCTOR – GENETIC COUNSELLING

INTRODUCTION

Modern medicine, increasing affluence and effective community health measures, have produced a change in the spectrum of incidence of human disease. Problems of malnutrition and infective disorders have receded in the background, giving place to an increased awareness of genetic or inherited diseases. Today, a **medical geneticist** – a physician specializing in the study of genetic disorders — is called upon to give advice to an individual, to families and even to entire governments on matters pertaining to the causation, incidence and risks of recurrence of inherited defects. Radiation hazards, newer drugs including the anti-cancer compounds and the means of biological warfare like mutant viral strains, would all fall within the purview of the **genetic counsellor.** The specific fields of activity in preventing, diagnosing and treating genetic problems are outlined in the text. The time is not far, when a medical geneticist could be the key figure in all teaching medical institutions in this country.

* * *

Genetic counselling deals with the problem of giving advice to families having, or likely to have, children with genetic disorders.

The causes of diseases can be arranged along a spectrum ranging from the purely genetic to the entirely environmental. (Table 24). With the control of microbial and deficiency diseases, the physician now more often encounters hereditary disorders and congenital abnormalities in practice.

TABLE 24: CAUSATION OF HUMAN DISEASES

Genetic	Genetic and Environmental	Environmental
Inborn errors of metabolism e.g., Phenylketonuria, Galactosemia Duchenne muscular dystrophy	Pyloric stenosis Club-foot Congenital dislocation of hip Diabetes mellitus	Tuberculosis, Lead poisoning, Pellagra, Accidental trauma
Rarer, simple Mendelian (Unifactorial) inheritance High risk of recurrence	*Commoner, Genetics multifactorial. Low risk of recurrence*	

A number of surveys has indicated, that at least one in fifty newborns, has a major congenital abnormality, about one in hundred has a unifactorial disorder and about one in two hundred has a major chromosomal abnormality. The physician not only faces the problems of diagnosing and treating these disorders but is also called upon to guide on issues such as:

1. The risk of recurrence of a hereditary disease in a family;
2. The risks attending the progeny from consanguineous marriages;
3. Genetic basis in cases of abnormal sexual development, infertility, recurrent abortion and congenital malformations;
4. Problem of child adoption;
5. Cases of disputed paternity;
6. Risks of acquiring common diseases; and
7. Detection of carrier.

1. The Risk of Recurrence of a Hereditary Disease in a Family

The physician first establishes a precise diagnosis through clinical examination and investigation. He informs the parents about the prognosis and the possible modes of treatment. He explains to the parents the risk of recurrence. The decision whether or not to accept the risk is left to the parents.

Determining the risk

In those conditions where the mode of inheritance is simple (autosomal dominant, recessive, or X-linked) it must be emphasized that chance has no memory, i.e. if the risk of inheritance is 1 in 4 it holds good with every offspring irrespective of whether other offspring are affected.

a. **Autosomal dominant disorders.** For a person heterozygous for an autosomal dominant gene, the chances of any of his children being similarly affected are 50 per cent.

b. **Autosomal recessive disorders.** When both parents are heterozygotes, the chances of their having an affected child is 1 in 4. Of the three unaffected sibs of the affected child, two have a chance of being heterozygous and therefore carriers. The probability of having an affected child is the product of the probabilities of parents being carrier, multiplied by 1/4, i.e., if two cousins of an affected individual marry each other the risk of each child of theirs being affected is 1/4 × 1/4 × 1/4 (cousins have 1/4 genes in common; see Table 15) or 1 in 64.

c. **X-linked disorders.** The carrier of an X-linked disorder is invariably a female. She transmits the gene to half her daughters who become carriers, and to half her sons, who get affected. In severe X-linked disorders, the affected males usually do not live long enough to beget children. If an affected male does have children, his sons are not affected, but all his daughters become carriers.

TABLE 25: RECURRENCE RISK OF DOWN'S SYNDROME DUE TO VARIOUS CHROMOSOMAL ABERRATIONS

Karyotypes			Chance of Recurrence %
Patient	**Father**	**Mother**	
Trisomy-21	normal	normal	1
Translocation			
21/21	carrier	normal	100
	normal	carrier	100
D/D	normal	carrier	10
	carrier	normal	5
21/22	normal	carrier	7
	carrier	normal	<3

If a woman has only one son affected, it can be due to a new mutation in the ovum. Under such circumstances, mutation is unlikely to recur and so her future children are unlikely to be affected. However, it is difficult to recognize a carrier, and to prove with certainty that a woman is not a carrier because of the inactivation of the X-chromosome.

It is important to distinguish between an autosomal recessive trait and an X-linked trait. In autosomal recessive inheritance, the normal sister of an affected male, even if she is a carrier, is unlikely to have affected children as the chances of her marrying another carrier are less when the trait is rare. In an X-linked trait, the sister of an affected male (because of their mother being a carrier) has 50 per cent chances of being a carrier and therefore the chances of any of her sons being affected are 1 in 4. The demonstration by appropriate tests that both the parents of an affected individual are carriers of the mutant gene, indicates that the trait is autosomal recessive, and not X-linked.

d. **Sporadic case of a hereditary abnormality.** When normal parents have a child with a rare congenital abnormality and there is no history of anyone else being similarly affected on either side of the family, the following must be considered to calculate the risk of the same abnormality affecting future children:

i. To rule out the possibility of the effect of a **teratogenic agent.** The chances that the abnormality will recur in future children will depend on the particular cause.

ii. To find out the possibility of a **new mutation** in the gametes of one of the parents. If mutation is for an autosomal dominant trait, it will be manifested in the offspring. If both parents are normal and the autosomal dominant trait is fully penetrant, then a new mutation is a likely cause. In that case, the chance of recurrence in subsequent children is very small.

iii. In an autosomal recessive disorder, the demonstration of **heterozygosity** in the parents by appropriate tests would confirm the basis. The **consanguineous marriage** of parents would offer supporting evidence.

iv. **Known X-linked recessive disorder.** Female children with normal sex chromosomes are not affected by an X-linked recessive disorder. The distinction should be made from an autosomal recessive disorder. The distinction is made on the basis of clinical examination as well as on the relative incidence of two forms of the disease. The distinction may not be drawn, in order to know the chances of further children being affected, but to advise the sister of an affected male as to whether her children would be similarly affected.

v. A number of syndromes are associated with specific chromosomal abnormalities, which tend to be sporadic with no one else in the family being affected. The examples of such syndromes are **Down's syndrome,** the **13-trisomy syndrome,** the **18-trisomy syndrome,** and the **cri du chat syndrome.** When abnormalities involve more than one system, e.g., nervous system, cardiovascular system and others, it is advisable to study the chromosomes of the affected child and of the parents, in order to find out if one of the parents carries a translocation. If the parents have normal chromosomes, chances are that the future children would not be affected by the same abnormality.

vi. For a multifactorial trait, the cases occur sporadically and the risk of recurrence is low.

During genetic counselling, the phenomenon of **genetic heterogeneity** (p. 94) should be kept in mind.

2. The Risks Attending the Progeny from Consanguineous Marriages

For normal parents the actual risks are small, though studies have shown that among the offspring of consanguineous marriages, there is an increased postnatal mortality rate and an increased frequency of congenital abnormalities and mental retardation.

However, if there is history of a disorder such as **phenylketonuria** in the family, the chance of first cousins carrying the same gene is 1 in 3; thus the chance of two cousins having an affected offspring is much more than in the case of unrelated parents. For example, if a person, with a phenylketonuric father, gets married to his first cousin from the father's side, the chance of their having an affected child is about 1 in 12 whereas the chance of two unrelated persons having an affected child is about 1 in 10,000. The above calculations are arrived at as follows. The person under consideration would be a **carrier.** The chance of his or her cousin being a carrier would be 1/3, the chance of the cousin's parent being a carrier being 2/3. The chance of any child of such a cousin marriage being affected would be $1\times1/3\times1/4=1/12$. Since the frequency of carriers of phenylketonuria in the general population is about 1 in 50, the chance that two unrelated persons will have an affected child is $1/50\times1/50\times1/4=1/10{,}000$.

Marriages between cousins carry not only an increased risk of producing a child homozygous for a detrimental recessive gene, but also of producing a child with a disorder due to more than one gene. There is also some evidence that the death rate in childhood, apart from known genetic or congenital conditions, is also raised. However, for the individual couple (in the absence of any history of recessive, or possibly recessive defects in the family) the increased risk is a very small one in absolute terms. Where a high rate of first-cousin marriages has been a long-established tradition in a community, it is self-evident that the vast majority of such marriages produce healthy normal children. There is also some evidence that toxaemia of pregnancy is less frequent when the parents are consanguineous.

3. *Genetic Basis in some Disorders*

In cases of delayed and **abnormal sexual development, infertility, recurrent abortion** and in individuals with congenital malformations, chromosomal studies help genetic counselling.

Chromosomal studies can reveal or rule out (*i*) **Turner's syndrome** in cases of delayed or abnormal sexual development in females; (*ii*) **Klinefelter's syndrome** or mosaicism in males when there is no apparent endocrinological or anatomical cause for sterility; (*iii*) translocation in one of the parents in cases of spontaneous abortion where the cause is not found; (*iv*) **Down's syndrome** in individuals with congenital malformations.

4. *Problems of Child Adoption*

Adoption agencies often want to know the risk of inherited disorders occurring in a child, when there is a history of some hereditary disorder in the child's background. The probabilities are calculated in a manner similar to the one discussed earlier under risk of recurrence of hereditary diseases in a family (p. 135). The main difficulties are encountered when there is a family history of a disease, which is not recognizable clinically or biochemically in the earlier years of the child's life e.g., Marfan's syndrome, Huntington's chorea.

5. *Cases of Disputed Paternity*

Paternity can not be proved with certainty, but it can be disproved without doubt. If the child has a blood group substance which could not have resulted from either of the parents, then the putative father is not the true father. Equally, if the child lacks a blood group which a putative father would positively transmit to all his offsprings, then the putative father cannot be the true father.

6. *Risks of Acquiring Common Disease*

Some of the common conditions such as cleft lip, cleft palate, idiopathic epilepsy, pyloric stenosis, idiopathic scoliosis, spina bifida, early onset of diabetes mellitus, etc., have no simple mode of inheritance. Some of these conditions are heterogeneous and include a number of etiologically different disorders; others are either due to many genes or due to the effect of environment. In such conditions only **empiric risks of recurrence** can be given. An **empiric risk** is defined as the probability of occurrence of a specified event based upon prior experience and observations rather than on prediction by a general theory. Empiric risks are calculated by estimating the frequency of the condition in the relatives of the affected persons.

In **multifactorial disorders,** the rate of recurrence in first-degree relatives is equal to the square root of the prevalence in the general population. As the prevalence frequency of most of the multifactorial disorders is between 1 in 500 and 1 in 2000, the recurrence rate usually ranges from 2 to 5 per cent.

7. *Detection of Carriers*

This is an important facet of genetic counselling as detection of carriers would remove uncertainty associated with various hereditary disorders. It is not possible at present to detect carriers in all conditions and with a high degree of certainty. The following methods are utilized for detecting carrier states:

i. Presence of some **clinical manifestation,** e.g., X-linked ocular albinism.

ii. Demonstrating **linkage** between a hereditary disorder and one of the marker traits, *e.g.*, linkage between one form of congenital cataract and the Duffy blood group locus.

iii. Demonstration of detectable **biochemical abnormalities** in carriers, *e.g.*, low levels of enzyme in acatalasia, elevated serum levels of creatine kinase in carriers of Duchenne muscular dystrophy.

Table 26 gives X-linked disorders where a carrier can be detected. **Carrier detection** is important when X-linked conditions are under consideration. Carrier detection is less significant in rare autosomal recessive traits. With common diseases due to recessive traits, detecting carriers can be useful, as such an individual can be warned of the risk of having affected children if he/she were to marry another carrier.

Table 27 gives methods for antenatal diagnosis.

1. *Transabdominal amniocentesis* (Fig. 78)

Transabdominal amniocentesis in combination with ultrasonography is the most widely used technique for prenatal diagnosis.

The risks involved in amniocentesis are foetal death, amnionitis, foetal puncture, amniotic fluid leakage and maternal vaginal bleeding.

2. *Radiography*

Besides a plain X-ray, **amniography** and **fetography** are also carried out. Amniography is a visualization of the foetus by introducing water soluble contrast dye into the amnion. Some of this contrast material is swallowed by the foetus and thus its gastrointestinal tract is outlined. This helps to diagnose gastrointestinal atresias and diaphragmatic hernias.

Fetography employs an oil soluble contrast material which adheres to the foetal *vernix caseosa* and permits visualization of the foetal outline. It is useful in the recognition of limb length anomalies or other major malformations which are externally manifested.

3. *Ultrasonography*

Ultrasonography is useful in (1) verifying whether the foetus is alive, (2) determining gestational age by measurement of the foetal head size, (3) diagnosing multiple gestations,

TABLE 26: CARRIER DETECTION TESTS IN X-LINKED DISORDERS

Disorder	**Abnormality**
Anhidrotic ectodermal dysplasia	Sweat pore count reduced
Becker muscular dystrophy	Serum creatinine kinase raised
Congenital agammaglobulinemia	*in vitro* immunoglobulin synthesis by lymphocytes reduced
Duchenne muscular dystrophy	Serum creatine kinase raised
Diabetes insipidus	Urinary concentration reduced
G6PD deficiency	Erythrocyte G6PD reduced
Haemophilia A	Factor VIII reduced
Haemophilia B	Factor IX reduced
Hypogammaglobulinaemia	IgG level reduced
Ocular albinism	Patchy depigmentation of retina and iris
Retinitis pigmentosa	Peripheral pigmentary changes in retina, Abnormal ERG or Fluorescein angiography
Vit. D-resistant rickets (hypophosphatemia)	Serum phosphorus reduced
X-linked ichthyosis	Corneal opacities present, Steroid sulphatase reduced

TABLE 27: METHODS FOR PRENATAL DIAGNOSIS

Invasive methods	
Amniocentesis	1. cell free fluid analysis 2. study of uncultivated cells 3. study of cultivated cells
Placentocentesis	
Fetoscopy	1. foetal visualization 2. foetal blood sampling 3. foetal skin biopsy
Amniography	
Fetography	
Non-invasive methods	
Biochemical analysis of maternal serum and urine	
Radiography	
Ultrasonography	

(4) determining placental and foetal positions, and (5) in detecting gross foetal malformation such as anencephaly or placental malformation such as a hydatid mole.

Table 28 gives abnormalities which may be detected by ultrasonography.

TABLE 28: ABNORMALITIES DETECTABLE BY ULTRASONOGRAPHY

Anomalies of Amnion
Hydramnios
Oligohydramnios
Anomalies of CNS
Anencephaly
Encephalocele
Hydrocephalus
Meningomyelocele
Spina bifida
Anomalies of chest
Diaphragmatic hernia
Intrathoracic cyst
Pulmonary hypoplasia
Small chest wall
Anomalies of abdomen
Duodenal atresia
Oesophageal atresia
Omphalocele
Polycystic kidney
Renal agenesis
Urethral obstruction and hydronephrosis
General body
Osteogenesis imperfecta with *in utero* fractures
Short limbed dwarfism syndromes

4. *Use of fetoscope*

This is not used in everyday practice. The technique allows collection of foetal blood by inserting a needle into the umbilical vessels under direct vision, as well as direct visualization of the foetus. It permits detection of external malformations. Obtaining of the blood sample allows prenatal detection of a number of disorders such as **haemophilia** and **Duchenne muscular dystrophy** which are not detectable in cell culture, but are noticeably expressed in blood even in a 16-week foetus.

5. *Placentocentesis*

The placentocentesis technique is for obtaining a foetal blood sample by inserting a needle into the placenta. This is usually performed at 18 weeks of gestation. This procedure is not commonly done as it carries the hazards of several serious complications.

Treatment of Genetic Disease

The vast majority of **unifactorial disorders** are serious, none is curable and relatively few are treatable. The principal approach to the control of genetic disease is, therefore, prevention through genetic counselling, with prenatal diagnosis and selective abortion, where possible.

Table 29 gives possible modes of management of some of the genetic disorders.

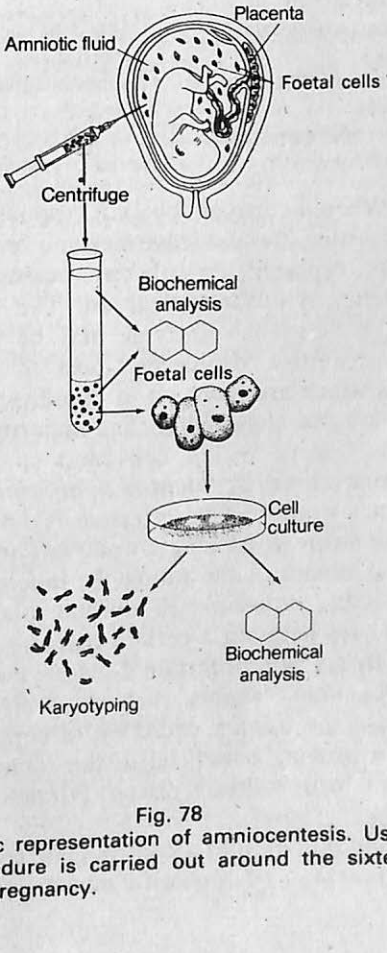

Fig. 78
Schematic representation of amniocentesis. Usually the procedure is carried out around the sixteenth week of pregnancy.

TABLE 29: EXAMPLES OF VARIOUS METHODS FOR MANAGING GENETIC DISEASE

Therapy	Disorder
Replacement of deficient protein	
antihaemophilic globulin	Haemophilia
Replacement of deficient vitamin	
vitamin D	vit. D resistant rickets
Replacement of deficient product	
cortisone	adrenogenital syndrome
thyroxine	congenital cretinism
Substrate restriction in diet	
phenylalanine	phenylketonuria
carbohydrate	galactosemia
Drug therapy	
insulin	diabetes mellitus
Preventive therapy	
avoidance of certain drugs	G6PD deficiency, porphyria
Injection of Rh gamma globulin	Rh incompatibility
Removal of diseased tissues	
splenectomy	hereditary spherocytosis

1. When an enzyme block is responsible for the disorder, the defective enzyme or protein may be replaced, its substrate restricted, or the deficient product replaced. The **replacement** of deficient coenzyme may be done in some disorders. However, most of the enzymes which are involved in hereditary disorders, are not identifiable. The majority of the enzymes work within cells and so even if enzymes were to get identified, injection of the enzymes would not be effective. Transplantation of tissue possessing normal enzyme activity is a dream of the future. In mucopolysaccharidoses, infusion of normal plasma or leucocytes helps to a certain extent.

2. **Drugs** help in certain diseases. For example, chelating agents such as penicillamine increase the urinary excretion of copper, and so are proving beneficial in the treatment of patients with Wilson's disease (Hepatolenticular degeneration).

When mutation of a control gene concerned with synthesis of a specific enzyme results in switching off the enzyme synthesis, drug therapy might be effective in inducing enzyme synthesis. For example in congenital nonhaemolytic jaundice, small doses of phenobarbitone can induce synthesis of the enzyme glucuronyl transferase.

3. **Genetic engineering by viral therapy.** It has been suggested that since Shope papilloma virus of rabbits induces the synthesis of the enzyme arginase without having any adverse effect when injected into humans, it may form an effective treatment for argininemia.

4. **Preventive therapy.** Avoidance of harmful drugs by individuals with hereditary disorders such as porphyria or G6PD deficiency, or prophylaxis for preventing sensitization by Rh-antigen are important examples.

5. **Surgical removal of diseased tissue.** Colectomy in polyposis coli, and splenectomy in hereditary spherocytosis are important examples of this mode of treatment.

6. **Transplantation of normal tissues**. This remains a future possibility.

TABLE 30: FREQUENCIES OF SOME METABOLIC DISORDERS AND CONDITIONS DETECTED BY SCREENING OF THE NEWBORN

Disorder or condition	Frequency	Screening involved
Phenylketonuria	1:13,000	Newborn blood
Galactosemia	1:50,000	"
Maple syrup urine disease	1:170,000	"
Homocystinuria	1:200,000	"
Hereditary tyrosinemia	Very low	"
Cystinuria	1:8,000	Newborn urine
Iminoglycinuria	1:12,000	"
Histidinemia	1:20,000	"
Hartnup disease	1:25,000	"
Methylmalonic aciduria	1:50,000	"
Cystathioninuria	1:70,000	"
Argininosuccinic aciduria	1:90,000	"
Hyperglycemia, nonketonic	1:180,000	"
Hyperprolinemia	1:200,000	"

Genetic Screening of the Newborn

Genetic screening of the newborn is mainly carried out to detect **inborn errors of metabolism.** The aim is the earliest possible recognition of defects in order to execute timely intervention to prevent the more serious consequences of a disorder. The screening is carried out by examination of (1) prenatal (maternal) blood (2) cord blood (3) newborn blood and (4) newborn urine. In addition to tests for classical inborn errors of metabolism like phenylketonuria, genetic screening of the newborn is also carried out for genetic disorders such as congenital hypothyroidism, haemoglobinopathies, α -antitrypsin deficiency, cystic fibrosis, Duchenne muscular dystrophy, hyperlipidemia, adenosine deaminase deficiency and congenital adrenal hyperplasia.

Table 30 gives frequencies of some metabolic disorders and conditions detected by screening newborn blood and urine. With such low frequencies of the various disorders, the **expense** and **effort** involved in carrying out genetic screening as a routine procedure, remains a debatable issue. Moreover, although the screening tests are sensitive, they do give false negative results at times, so that a negative result from screening does not necessarily exclude the presence of the disorder.

SUMMARY

Genetic counselling refers to the giving of scientific advice under the following circumstances:

1. Risk of recurrence, in a family, of a hereditary disease – autosomal dominant 50% to 100%; autosomal recessive 1 in 4; X-linked disorder in male: male progeny – nil, female progeny – 100% carrier; X-linked disorders in female: male progeny – 50%, female progeny – carrier 50%.
2. Risk attending the progeny from consanguineous marriages: normal parents – small; parents with a history of a disorder – the risk increases manifold in recessive disorders.
3. Determining genetic basis in cases of abnormal sexual development, infertility, recurrent abortion and congenital malformations.
4. Problem of child adoption – determining the risk of inherited disorders in child.
5. Cases of disputed paternity – paternity can be often disproved on the basis of blood groups of child and putative father.
6. Risks of acquiring common disease – in conditions due to many genes or due to effect of environment, only an empiric risk of recurrence is given.
7. Detection of carriers based on some clinical manifestations or demonstrations of detectable biochemical abnormalities.

Antenatal diagnosis of genetic disease

Procedures: 1. Transabdominal amniocentesis – amniotic fluid aspirated through abdominal wall. Cytological and biochemical study of amniotic fluid permits detection of gross anomaly. Procedure carried out at about 16 weeks of gestation. 2. Radiography 3. Ultrasonography – to determine (a) viability of foetus (b) gestational age (c) multiple gestations (d) placental and foetal positions (e) gross foetal malformations 4. Fetoscopy 5. Placentocentesis.

Treatment of genetic disease

1. Replacement of deficient enzyme, protein, etc.
2. Drugs
3. Viral therapy
4. Prevention – avoiding harmful drugs
5. Surgical removal of diseased tissue
6. Transplantation of normal tissue.

Genetic screening of the newborn is carried out to detect inborn errors of metabolism by examination of maternal blood, cord blood, blood and urine of newborn.

QUESTIONS

1. State and explain the various aspects of genetic counselling.
2. Write a note on the methods for treating genetic disorders.
3. Write notes on:
   Carrier
   Empiric risk
   Amniocentesis
   Fetoscopy
   Prenatal diagnosis

10 EXPERIMENTAL GENETICS

INTRODUCTION

Human genetics has largely been studied by means of **pedigree analysis** and collection of data regarding inherited traits in populations and then subjecting the data to **mathematical** and **statistical analyses.** Examples in point are the haemoglobins, the blood group systems, the inborn errors of metabolism and the study of gene frequencies for other genetic disorders. Recent years have, however, seen spectacular progress in this field as a result of the extrapolation of data from experimental genetics to human heredity. Two factors have been responsible : (1) The realization that the genetic material is almost universally the same in living organisms and (2) the recent technological advances which have made possible the direct manipulation of genetic material in and in between various living forms. The extensive **human chromosomal maps** and the concept and reality of **gene libraries** would not have been possible without the modern experimental techniques.

Since a plethora of laboratory techniques has been brought to bear on a bewildering array of life forms, it is not feasible, in a book of this size, even to enumerate the permutations and combinations of **cell-hybridization** and **DNA-recombinant** techniques.

The fields, which find a brief mention here are those which have perhaps contributed the most to the understanding of fundamental genetic principles, and are likely to contribute to human medical genetics in the near future.

* * *

The understanding of genetics has largely come from experiments on micro-organisms, drosophila and some laboratory animals. Man is ill-suited for experimental work in genetics due to his prolonged generation time, limited family, and nonfeasibility of test matings to acquire desired information or to test hypotheses. Though man is neither a molecule nor a mouse, it is observed that at the molecular level all living organisms have similar genetic mechanisms and therefore the knowledge obtained from experiments on lower organisms can often be applied to man.

A brief account of the important experiments and observations, which have laid the foundations of the science of genetics, is given here.

1. Mendelian Genetics

Mendel experimented with **garden peas** *(Pisum sativum)*. Seven contrasting traits were chosen for study. Vines were either tall or dwarf; unripe pods were green or yellow, and inflated or constricted between the seeds; flowers were either distributed along the stem (avial) or bunched at the top (terminal); nutritive parts of ripe seeds were green or yellow; the outer surface of the seed was smooth or deeply wrinkled; and the seed coats were either white or gray.

Mendel classified the plants resulting from the crosses, and meticulously recorded the data. Based on these data, he propounded the laws of heredity. Mendel's results and laws can now be rationally explained in terms of the behaviour of chromosomes and genes.

2. Studies on Drosophila melanogaster

Drosophila has been and perhaps still is the most widely used single material for original investigations, and learning exercises for students of genetics. The work of Morgan and his collaborators with *D.melanogaster,* a variety of fruit fly, is of historic importance. The studies revealed that the Mendelian law of **independent assortment** was not universally applicable. Morgan demonstrated the principle of **linkage** by showing that all genes in *D. melanogaster* were clustered into **four linked groups,** corresponding to the four pairs of chromosomes. The first chromosome has many hundreds of genes. The fourth chromosome is the smallest and has only a a few. The linkage can be broken by recombination during the meiotic prophase. Other principles of genetics which emerged from studies on Drosophila are listed below.

(*i*) Genes as modifiers to alter the visible effects of other genes (an *E-S* enhancing the effect of the dominant gene *S* for the star eye)

(*ii*) Sex determination (e.g., a gynandromorph resulting from irregularity in early cell cleavage or from hormonal disturbances influencing expression of genes)

(*iii*) Sex linkage (the first experimental evidence of sex linkage came in 1910 with the discovery by Morgan of the white-eyed male mutant drosophila in red-eyed flies)

(*iv*) Linkage and linkage maps

(*v*) Crossing over

(*vi*) Mutations (e.g., production of hereditary abnormality called Notch wing by radiation)

(*vii*) Multiple alleles

(*viii*) Various chromosomal aberrations

(*ix*) Mapping of chromosomes.

The most detailed insight into the linear arrangement of specific entities along the length of chromosomes has been obtained from **giant chromosomes of the salivary glands** of the larva of the fruit fly (Fig. 79). These chromosomes, instead of looking like threads, look like wide cylinders marked by many crossbands or discs. Chromosomal mapping may also be done by means of a deficient chromosome. In *D. melanogaster,* X-rays can permanently remove sections anywhere from the chromosomes. The remaining end pieces may fuse at their breakage points, resulting in a chromosome with a deletion. For example, some of the offsprings of an X-rayed fly may have an abnormality called Notch wing (Fig. 80) where, instead of the wings having smooth and continuous outlines, they look as if notches had been cut out of them. The **Notch character** is transmitted to later generations. Study of chromosomes of such flies show absence of a specific region in a specific rod-shaped chromosome. This illustrates that the missing part of the chromosome has a "gene for normal wing outline," or a "gene for non-Notch wings."

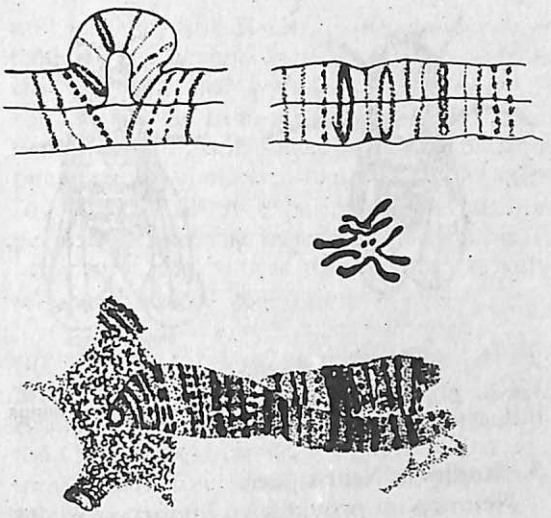

Fig. 79

Chromosomes of *Drosophila melanogaster* (central figure) from an ovarian cell. Cells of the larval salivary gland, however, have giant chromosomes. The lower figure shows one such pair, which corresponds to the two dots of the ovarian cell chromosome pair, at the same magnification.

Notch deficiencies in the chromosomes of two different strains of *D. melanogaster* (upper left and right). The lower members of the homologous pairs show the deficiency. This type of deletion results in notching of wings.

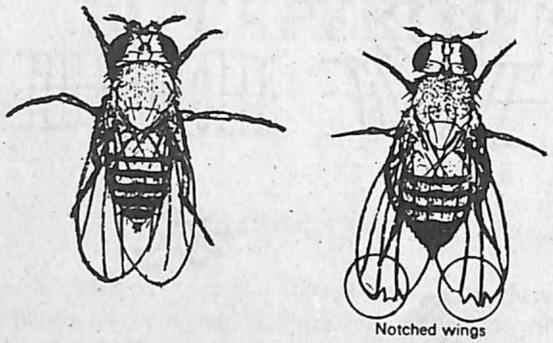

Fig. 80
Female *Drosophila melanogaster* with hotched wings. Normal female on the left.

3. Studies in Neurospora

Neurospora provides an important model for study in genetics. Mold neurospora is ideal for study of **recombination** and its relationship to the biochemical expression of genes. In the spore mother cells, after the first and second meiotic divisions, there is a mitotic division and thus, in all, eight ascospores are produced. Each ascospore is haploid and contains a single chromatid; genetic recombination can be studied by separating and culturing each ascospore. It is possible to identify and to follow the fate of each of the four chromatids present in the meiotic (maternal) chromosome and thus to determine how many chromatids are involved in crossing over.

4. Studies on somatic cells

The experimental studies on somatic cells help in obtaining details on genetic linkage. In these experiments, cells from two different species are fused with the help of a virus, and then cultured. The resulting alterations in the chromosomes and the enzymes are studied (Fig. 81). For example, mouse cells unable to synthesize thymidine kinase, were fused with human cells which could synthesize the enzyme thymidine kinase. After several generations all the human chromosomes were lost from the hybrid cells with the exception of chromosome 17. The hybrids also retained the ability to synthesize thymidine kinase. This indicated that the ability to synthesize the enzyme was located on chromosome 17.

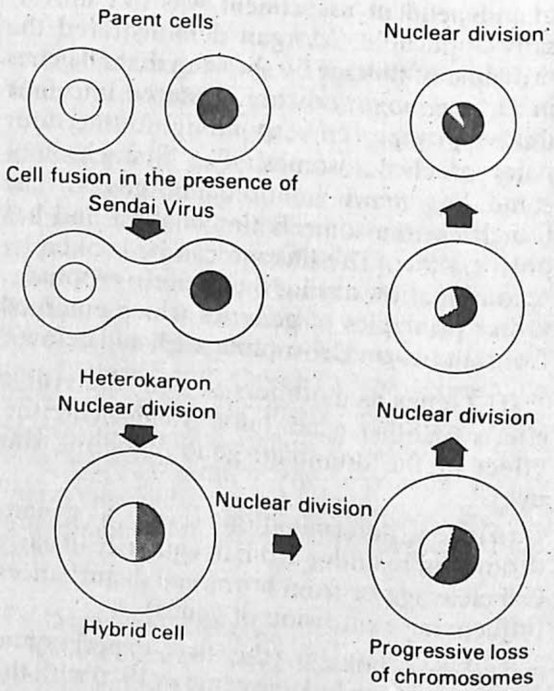

Fig. 81
Scheme showing cell hybridization and subsequent selective loss of chromosomes from the hybrid cells.

5. Studies on Microbes

Experiments on microbes have many advantages such as exceedingly short generation time, the ease of manipulation and the direct relationship between the gene and its effect. **Bacteria** and **viruses** are most often used in the studies. **Microbial genetics** has thrown light on mechanisms of heredity at the molecular level – the fact most emphasized in genetics at present.

Bacteria are usually haploid, multiply by

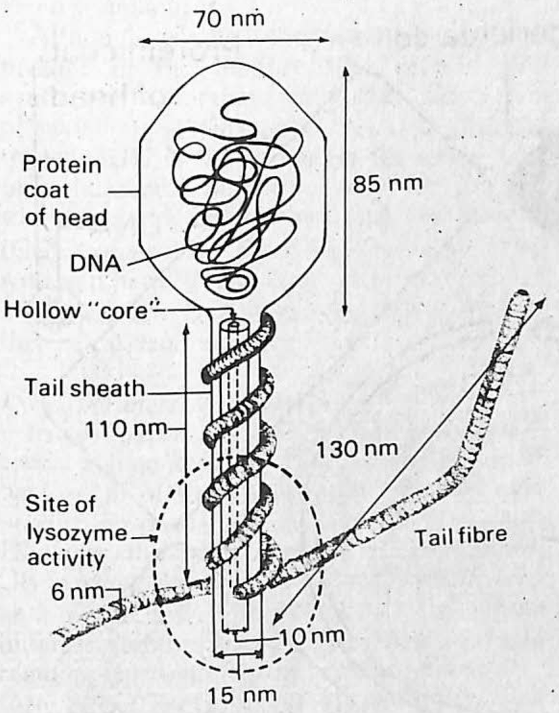

Fig. 82

Diagram showing the molecular organization of the T4 bacteriophage.

simple division, and transmit hereditary characteristics in this way. **Bacteriophages** are the organisms best suited for analysis of the fine structure of genes. Fig. 82 shows the complex molecular structure of a T4 phage. A bacteriophage is a haploid organism having a single chromosome. A bacteriophage injects its DNA molecule into a bacterium and multiplies, producing hundreds of progeny in a matter of minutes. The crowding brings about lysis of the bacterium. Some celebrated experiments, mentioned below, have thrown light on the basic tenets of molecular genetics.

A. Experiments which proved that DNA is the genetic material

(i) Bacterial transformation: The Griffith experiment. The pneumococcus, a causative organism for pneumonia exists in two phenotypes. S form – smooth, encapsulated, and virulent, and R form – rough, unencapsulated, and benign. In 1928, Griffith showed that mice infected with live, non-virulent R form would die from pneumonia if also given heat-killed S form bacteria. Live S form pneumococci could be isolated from the mice so killed. Griffith experiment proved that **permanent bacterial transformation –** from R form to S form – was transmissible to subsequent bacterial generations.

(ii) Bacterial transformation: The Avery-MacLeod-McCarty experiment. In 1944, Avery and coworkers demonstrated that in the Griffith experiment, it was DNA that was transferred from dead S to live R. They achieved this by adding to R form pneumococci, extract containing DNA from S form instead of dead S form pneumococci. The control experiment, using the same extract (live R form × DNA from S form) to which the enzyme DNAase had been put to destroy the DNA, showed that destruction of DNA in the extract,took away its ability to transform R form to S form. This proved that some DNA of S form had been incorporated into the genetic material of R form, bringing about a permanent transformation.

(iii) Bacteriophage reproduction: The Hershey-Chase experiment (Fig. 83). T4 phage was grown in two kinds of culture media, one containing radioactive phosphorus (P^{32}) which selectively gets incorporated into DNA and not into protein; and the other containing radioactive sulfur (S^{35}) which gets incorporated into protein but not into DNA. This produced two types of phage, one with the radioactive label in its DNA and the other with label in its protein coat. When DNA-labelled phage was grown on *E.coli* with unlabelled-DNA the label appeared inside the *E.coli.* However, when the protein-labelled phage was grown with unlabelled *E.coli,* the label remained outside the *E.coli.* This illustrates that the material which entered the cell and was responsible for the reproduction of phages was DNA, the protein having no

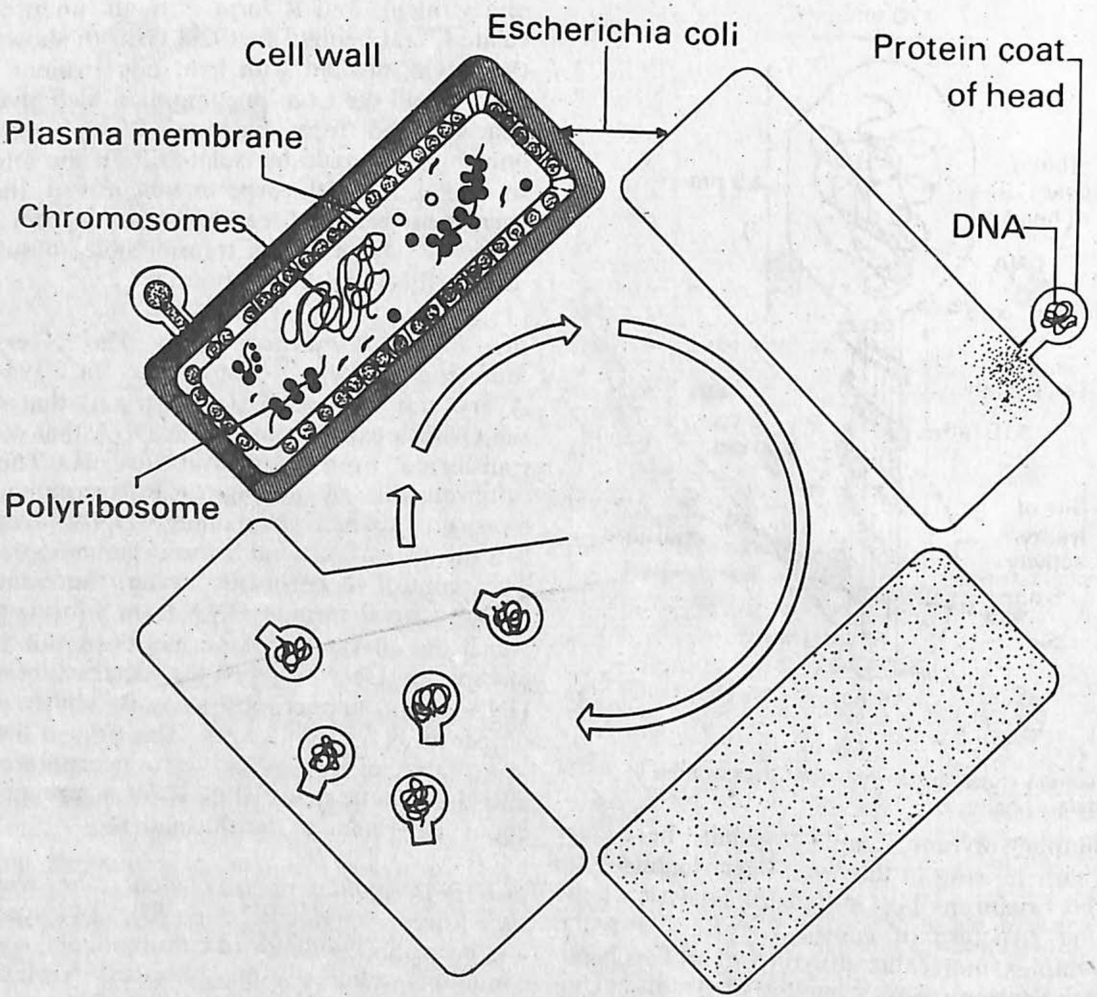

Fig. 83
Scheme showing the result of an attack by a bacteriophage on the *E. coli* bacilus. Details of *E. coli* organization shown.

role to play in phage reproduction.

B. Studies to show structure of DNA

In 1953, on the basis of X-ray diffraction studies of DNA, Wilkins, Watson and Crick proposed a structure of the DNA molecule fulfilling all the essential requirements of being a sufficiently versatile structure providing great variety of different genes, with the ability to reproduce itself in such a manner that an identical replica of itself could be reproduced.

C. Studies involved in cracking of the genetic code

Nirenberg and Matthaei in 1961 first made the observation that synthetic polyribonucleotides could act as artificial mRNAs and could stimulate the incorporation of amino acids into polypeptides. They added RNA containing only the pyrimidine (nitrogenous base) uracil to mixture of amino acids, enzymes, and ribosomes, and were able synthesize a simple protein. Thus they initiated a procedure *in vitro* to imitate the *in vivo* sequences.

Although all the other amino acids were present in the mixture, the protein they synthesized contained only the amino acid phenylalanine. This gave the clue that the triplet UUU is the codon for the amino acid phenylalanine. Similarly poly A (AAA) stimulated the uptake of lysine and poly C (CCC) of proline. By 1963, the experiments with synthetic RNAs done in laboratories of Nirenberg and Ochoa had established most of the codon sequences.

D. Experiments for synthesis of gene

In 1970, Khorana and his colleagues synthesized a gene *de novo*. They assembled the 77 base pairs of the gene which codes for the production of alanine transfer RNA in yeast. However, this artificial gene, though structurally correct, was non-functional both in cells and in the test tube because it lacked the initiator and terminator signals that start and regulate the synthesis of transfer RNA.

In 1973, Khorana and his coworkers succeeded in synthesizing the **first totally artificial gene** with 126-unit DNA segment which codes for the production of tyrosine transfer RNA in *E.coli.* The assembling of **126 nucleotides** in an exact order was done by first chemically synthesizing short deoxynucleotide sequences, which then were joined by hydrogen bonds to form complementary strands. It was followed by enzymatic synthesis to join the double-stranded pieces. The structural gene was made by joining of 26 pieces of single-stranded oligonucleotides, which first formed four double-stranded segments. These segments were joined to form the entire gene with the 126 pairs of complementary nucleotides.

In 1976, Khorana *et al.*, succeeded in synthesizing and joining the structural gene with its 56 nucleotides of the promoter region and 25 nucleotides of the terminator region. It was the addition of these segments that made the gene functional. The synthetic gene has been shown to correct a mutational defect in bacteriophage *lambda*, a virus that infects bacteria, by replacing a defective gene. The gene is also effective in test tube experiments.

E. Study of molecular structure of eukaryotic genes

Restriction endonuclease analysis

Restriction endonucleases are enzymes which recognize specific sequences of bases in **double stranded DNA molecules** and **cut DNA** wherever these specific sequences or restriction sites occur. Approximately one hundred different restriction endonucleases, each of which recognizes a unique sequence of bases, have been isolated. Individual **restriction enzymes** are used to splice DNA into reproducible and characteristic sets of fragments, several hundred to several thousand bases long, which are isolated according to length, by gel electrophoresis.

For a given sample of DNA containing a specific gene, it is the choice of the endonuclease that decides whether the restriction site for the endonuclease falls within the gene or outside it. If the gene does not contain the restriction site for a particular endonuclease, the entire gene will lie within the limits of the DNA fragment. If the gene itself contains one or more restriction sites for a particular endonuclease enzyme, the gene will then be present on DNA fragments of several different lengths.

Southern hybridization

When a sample of DNA is digested by a restriction enzyme and the fragments separated according to length, the fragments containing any particular gene are identified by the Southern hybridization (so named after the scientist) procedure. This technique is based on the principle that the sequence of bases in a gene and its messenger RNA are complementary and that under appropriate conditions, mRNA binds or hybridizes only with those DNA fragments, which share a highly complementary base sequence. To perform Southern hybridization, a piece of

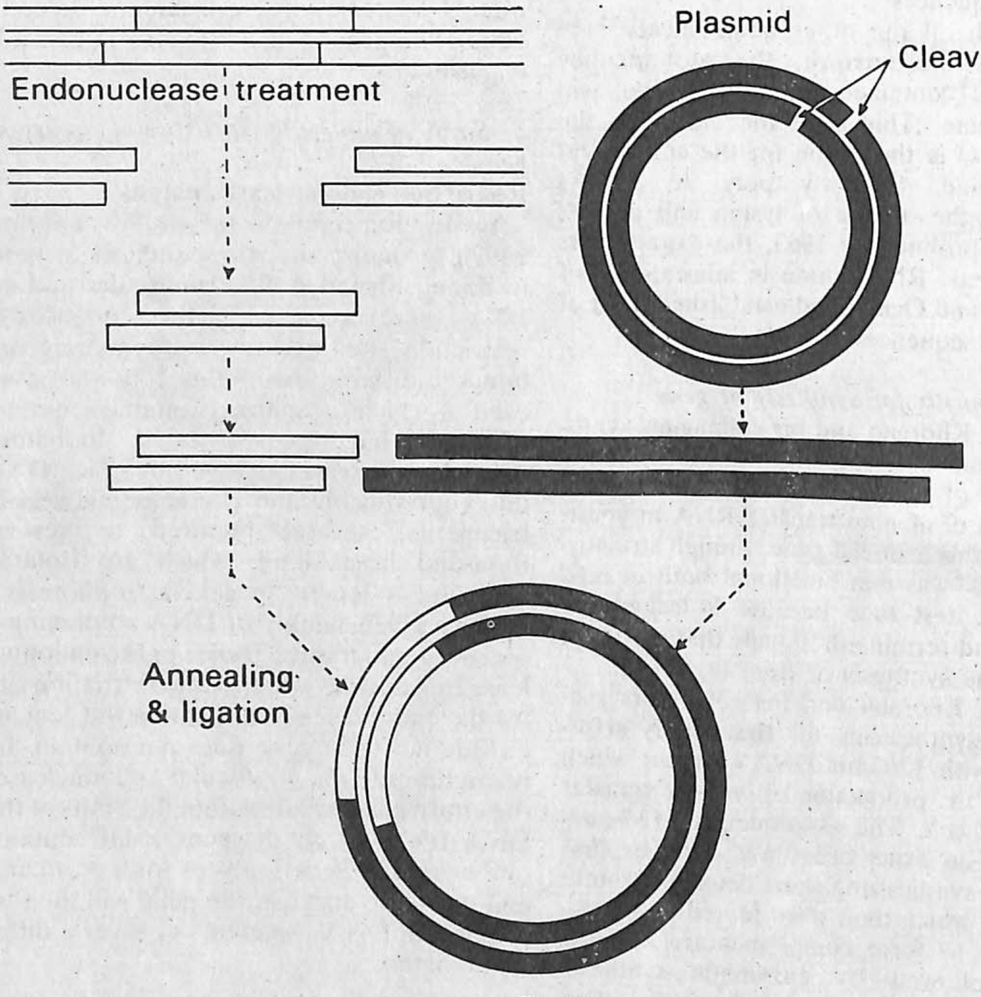

Fig. 84
Recombinant DNA cloning. DNA fragment, containing a gene of interest, is added to the plasmid DNA which is cleaved and therefore becomes linearized. The mixture is annealed and ligated to produce a series of circularized recombinant plasmid DNA molecules.

nitro-cellulose paper is first 'blotted' on to an electrophoretic gel containing the DNA fragments separated, according to their lengths, after having undergone an endonuclease digest. The DNA containing paper replica is then made to react with a specific radioactive mRNA, which hybridizes only with the complementary DNA fragments on the paper. The locations of these DNA-mRNA complements are identified by autoradiography. Thus the Southern hybridization technique allows DNA sequences of special interest to be located among hundreds or thousands of otherwise unresolvable fragments.

Recombinant DNA cloning

This technology is useful for inserting and replicating the gene in a bacterial cell and thus providing large amounts of the DNA fragment required for a detailed analysis of gene structure. In this technique, a small circular piece of DNA (a plasmid) is used as a vehicle or vector for inserting the desired gene into a bacterium. Plasmids are able to replicate independently in bacteria and remain separate from the bacterial chromosomes carrying the main set of bacterial genes.

The first step in the process involves treatment of the plasmid DNA with a selected restriction endonuclease, which cleaves the plasmid DNA at a single site opening the circle, but leaving the sequence of genes involved in plasmid replication intact. A segment of eukaryotic DNA, containing the gene of interest, is introduced into the plasmid, where it is sealed in place by another enzyme, DNA ligase. The newly formed **recombinant molecule** is then introduced into host cells, where it can replicate. A **host cell** thus **transformed** is then cloned in order to produce enough material under study (Fig. 84).

The DNA fragment carrying the gene of interest can be produced in the laboratory as follows: Messenger RNA can be obtained from the cells rich in them. For example, reticulocytes are rich in haemoglobin messenger RNA. Complementary DNA is made on mRNA template by means of reverse transcriptase.

The possibilities for useful application of recombinant DNA lie in **genetic engineering** to cure genetic disorders caused by lack of a specific enzyme; and for the industrial manufacture of hormones for medical use, While the above mentioned possibilities can be hoped for, there are many potential hazards in such research.

DNA sequencing

Once a specific DNA fragment containing a gene has been cloned, the **entire sequence of the cloned DNA** can be deduced. The procedure involves the following steps: First, the DNA is digested with a variety of restriction endonucleases to create a series of **overlapping DNA fragments.** The resulting fragments are then separated according to size, isolated from the gel, and the base sequence of each isolated fragment determined. Since the sequences overlap, they can be put together to determine the complete DNA sequence of the cloned fragment.

SUMMARY

Mendel was a pioneer experimental geneticist — his *garden peas* observations are legendary. Morgan in the early part of this century, worked on *Drosophila melanogaster* (fruit fly) providing insights into genes as modifiers, and into sex determination, sex linkage, linkage and linkage maps, crossing over, mutations, multiple alleles, mapping of chromosomes, and chromosomal aberrations. The giant chromosome of the salivary gland of the fruit fly larvae helps in chromosomal mapping.

Modern experimental genetics rests upon the facts that the genetic material — DNA — occurs universally in almost all living forms; DNA can be extracted, experimented upon and studied in and outside micro-organisms, animals and humans. Its rewards are human chromosomal maps, gene libraries, cell-hybridization, recombinant-DNA, and so on. Experimental genetics has come to stay, and prosper.

Studies of mold (Neurospora) reveal the nature of crossing over and genetic recombination. Somatic cells from different species, hybridized through virus, allow localization of enzyme synthesis, say, on a particular chromosome. Microbial genetics permits analysis of the fine structure of genes — e.g., unravelling of molecular organization of T_4 bacteriophage. Bacterial transformation — from rough, uncapsulated, benign R form to smooth, encapsulated, virulent S form — as well as bacteriophage production are clearly attributed to DNA production/transfer/mutation.

Studies on DNA have travelled a long way from the time Watson and Crick proposed a viable and workable model of DNA on the basis of X-ray diffraction of DNA. Matthaei, Ochoa and others synthesized polyribonucleotides that could act as artificial mRNAs — the basis of the determination of the codon sequences.

Khorana and colleagues synthesized a totally artificial gene, making a multi-unit DNA segment, rendered functional, *in vitro* and in microbes, by tagging to it nucleotides of the promoter region and nucleotides of the terminator region.

The elucidation of the molecular structure of eukaryotic genes is owing to surgery on the genes being rendered possible by restriction-endonucleases, and hybridization techniques. Eukaryotic DNA can now be cut, spliced, and hybridized with the tools of recombinant genetic engineering, and DNA sequencing.

QUESTIONS

1. Write a note on experimental genetics.
2. State briefly the significance of:
   Mendel's experiments
   Studies on *Drosophila melanogaster*
   Somatic cell studies
   Studies on microbes
3. Describe briefly:
   The Griffith experiment
   The Avery-Macleod-McCarty experiment
   The Hershey-Chase experiment
4. Write notes on:
   Restriction endonuclease analysis
   Southern hybridization
   Recombinant DNA cloning
   DNA sequencing.

GLOSSARY

Acentric. A chromosomal fragment without a centromere.

Acrocentric. Indicates a chromosome or chromatid with a nearly terminal centromere making one arm very short.

Albinism. A condition characterized by congenital absence of pigmentation resulting in pale, milky skin, light hair and pink eyes.

Alleles, Allelomorphs. One or more alternative forms of a gene found at the same locus on homologous chromosomes in an individual and/or in a population of individuals. Alleles segregate at meiosis; a child thus usually receives only one of each pair of alleles from each parent. See also Multiple alleles and Isoalleles.

Allograft, Homograft. A tissue graft from a donor to a host with different genotype, host and donor being members of the same species.

Allotypes. Genetically determined differences in antigens.

Amino acid. Organic compound containing both basic amino (NH_2) and acidic carboxyl ($COOH$) groups. There are over twenty amino acids universally found. Amino acids are polymerized to form proteins.

Amniocentesis. Aspiration of amniotic fluid, by a syringe and needle, from the amniotic cavity through the abdominal wall and the uterus. The term is also applied to the entire procedure of antenatal diagnosis by culture and analysis of amniotic fluid cells.

Amorph. A gene having no effect; an apparently inactive gene.

Anaphase. Stage of mitosis or meiosis when daughter chromosomes are separating from the equatorial plate towards the opposite poles of the spindle.

Aneuploid. A chromosome number which is more or less than an exact multiple of the haploid number, e.g., $2N\text{-}1$ *or* $2N + 1$ where N is the haploid number of chromosomes.

Antibody, Immunoglobulin. A specific complex protein produced in response to the introduction of a specific antigen into an animal and reacting specifically with that antigen.

Anticipation. The apparent tendency for some diseases to begin at an earlier age and to increase in severity with each succeeding generation; apparently this has no biological basis.

Anticodon. A triplet in the tRNA complementary to an mRNA codon. (See codon).

Antigen. A substance which elicits antibody formation by immune competent cells and reacts specifically with the antibody so produced.

Ascertainment. The finding and selection of families with hereditary disorder.

Association. The occurrence of two or more phenotypic characteristics in members of a kindred or a population in a frequency greater than would be predicted on the basis of chance.

Assortative mating, Nonrandom mating. The preferential selection of a mate with a particular genotype. Preference for a mate of the same genotype is positive assortative mating; preference for a mate of a different genotype is negative assortative mating.

Assortment, genic and chromosomal. The random distribution to the gametes of different combinations of genes and chromosomes. Each $2N$ individual has a paternal and a maternal set of chromosomes forming N homologous pairs. At anaphase of the first meiotic division, one member of each chromosome pair passes to each pole, and thus the gametes contain one chromosome of each type, but this chromosome may be either of paternal or maternal origin. Thus, nonallelic genes assort independently to the gametes, Exception: linked genes.

Autograft. A graft from the host's own tissues.

Autoimmunity. Immunity to self, seen in certain conditions in which an individual forms antibodies against one or more of his own antigens.

Autosome. Any chromosome other than the sex chromosomes. In man there are 22 pairs of autosomes.

B cells. Small lymphocytes (bursa-equivalent cells) derived from precursors in the bone marrow sensitive to bacterial and viral antigens, and transformed by antigenic stimulation into plasma cells which produce humoral antibodies.

Back cross, Test cross. A mating of a heterozygote with a recessive homozygote (T/t × t/t) in which the progeny (1/2 T/t, 1/2 t/t) reveals the genotype of the heterozygous parent. The double backcross mating (T/t R/r × t/t r/r) is the most useful mating for linkage analysis.

Bacteriophage. A virus which infects bacteria.

Banding. The techniques of staining chromosomes in a characteristic pattern of cross bands, thus allowing identification of each individual chromosome pair. Giemsa banding (G banding) and quinacrine fluorescence banding (Q banding) are the best available techniques.

Barr body, Sex chromatin. The condensed single X-chromosome seen adjacent to the nuclear membrane in the nuclei of somatic cells of female mammals; named for M.L.Barr, who first described this body, thus demonstrating sexual dimorphism in somatic cells.

Base pairing. Adenine forms a base pair with thymine in DNA and with uracil in mRNA; similarly guanine pairs with cytosine in both DNA and RNA. This specificity of base pairing is fundamental to DNA replication, and to its transcription into mRNA for protein synthesis.

Bivalent. A pair of homologous chromosomes as seen during metaphase of the first meiotic division.

Blood group. The term is restricted to the genetically determined red cell antigens.

Burden. In clinical genetics the term burden refers to the total impact of a genetic disorder on the patient, his family and the society as a whole.

Carrier. An individual heterozygous for a single recessive abnormal gene which is not expressed phenotypically, though it may be detectable by appropriate laboratory tests.

Centric fusion. Fusion of the long arms of two nonhomologous acrocentric chromosomes at the centromere. Also known as Robertsonian translocation.

Centriole. One of the pair of organelles which form the points of focus of the spindle during cell division. Centrioles are short cylinders containing nine pairs of peripheral microtubules disposed about a central cavity. The centrioles lie together at right angles to each other outside the nuclear membrane at prophase, and replicate and migrate during cell division to opposite poles of the cell.

Centromere, Kinetochore, or Primary constriction. The small mass of heterochromatin within a chromosome by which the chromatids are held together, and by which a chromosome becomes attached to the spindle during cell division.

Chiasma. The cross configuration of chromatids of homologous chromosomes during the diplotene stage of the first meiotic division.

Chimera. An individual containing cells derived from different zygotes. In human genetics the term is used with reference to blood group chimerism, in which dizygotic twins exchange haematopoietic stem cells *in utero* and continue to form blood cells of both types; or to "whole-body" chimerism, in which two separate zygotes are fused into one individual.

Chromatid. One of the two daughter strands of a duplicated chromosome which are joined by a single centromere. Each is destined to form one chromosome in the daughter cell — one half of a replicated chromosome.

Chromatin. The nucleoprotein fibres the chromosomes are composed of.

Chromomere. A densely coiled region of chromatin on a chromosome. Chromomeres give the extended chromosome a beaded appearance.

Chromosomal aberration. An abnormality of chromosome number and/or structure.

Chromosomes. DNA-histone threads residing in the nucleus of a cell. They carry genetic information. Invisible as distinct entities during interphase, they become prominent during metaphase of cell division.

Cistron. The smallest structural and functional unit of genetic material (DNA) which specifies the formation of a particular polypeptide chain. The structural gene as usually conceived is synonymous with the cistron.

Clone. A group of genetically identical cells derived from a single cell by repeated mitosis.

Codominance. When both alleles are fully expressed in the heterozygote.

Codon. A triplet of three successive bases in a DNA or RNA molecule specifying a single amino acid.

Coefficient of inbreeding (F). This represents the probability that an individual has received both alleles of a pair from a common ancestral source or the proportion of loci at which he is homozygous. For example, offspring of a first cousin marriage. For any gene the father has, the chance that the mother also has the same, is one-eighth. For any gene the father gives to his child, the chance that the mother has the same gene and will transmit it is 1/2 × 1/8 = 1/16. This is the coefficient of inbreeding for the child of a first cousin marriage. It indicates that the offspring has a 1/16 chance of being homozygous at any given locus, or that he is homozygous at 1/16 of his loci.

Coefficient of relationship. Represents the probability that the two persons have inherited a certain gene from a common ancestor, or the proportion of all their genes that have been inherited from common ancestors.

Colinearity. The parallel relationship between DNA and a protein, in which the sequence of bases of DNA specifies the sequence of amino acids of the protein.

Complementation. It is the ability of two different genetic defects to correct for one another, thus establishing that the defects are not in the same gene, e.g., when two persons, each homozygous for a recessive defect, produce children without the defect. The term is more commonly applied to the complementation test, in which two mutant cell lines grown together mutually correct one another's biochemical defect or in which a somatic cell hybrid made from two genetically defective cells lacks the defects of the parent lines.

Compound. A heterozygous individual (or genotype) who possesses two diferent mutant alleles (not 'wild' or 'normal') at one particular locus on homologous chromosomes.

Concordant. Twins are said to be concordant for a given trait if both exhibit the trait.

Congenital. Any abnormality, genetic or otherwise present at birth.

Congenital trait. Trait present at birth, not necessarily genetic.

Consanguinity, Blood relationship, Genetic relationship. Consanguineous individuals have at least one common ancestor in the preceding few generations.

Coupling. In a heterozygote for two loci, both nonallelic genes of interest at different loci on the same chromosome are said to be linked in coupling or in *cis* configuration.

Crossover, Crossing over, Recombination. The exchange of genetic material between homologous chromosomes. The chiasmata seen at diplotene are the physical evidence of crossing over.

Cytogenetics. The science that combines the methods and findings of cytology and genetics, concerned mainly with the chromosomes and their correlations with the phenotype.

Degeneracy of the code, Degenerate code. The genetic code is said to be degenerate because more than one nucleotide triplet codes for the same amino acid.

Deletion. The loss of a segment of the genetic material from a chromosome, a form of chromosomal aberration.

Dermatoglyphics. The study of the patterns of the ridged skin on the palms, fingers, soles, and toes.

Dicentric. A structurally abnormal chromosome with two centromeres.

Dictyotene. The interphase-like stage in which the oocyte persists from late foetal life until ovulation. During this stage, the oocyte has not yet completed the prophase of meiosis I.

Diploid. The double state of all chromosomes in normal somatic cells (Symbol: 2*N*).

Discordant. Twins are said to be discordant with respect to a trait, if one shows the trait and the other does not.

Dispermy. Fertilization of a duplicated egg nucleus, or egg and polar body, by two sperms. This event can produce a rare type of chimera in which two separate zygotes fuse to form a single individual.

Dizygotic, Dizygous, Fraternal. Type of twins produced by the fertilization of two different ova by two different sperms. Dizygotic twins are no more similar genetically than are brothers and sisters.

DNA, Deoxyribose nucleic acid. The nucleic acid of the chromosomes, which carries the genetic code. Also found in the mitochondria.

Dominant. A trait which is expressed in individuals even when they are heterozygous for a particular gene.

Dosage compensation. The genetic mechanism by which the two X chromosomes of the normal female are rendered quantitatively identical, in their effect, to the one X chromosome of the normal male. See Lyon hypothesis.

Drift, Random genetic drift. Fluctuations in gene frequencies which tend to occur in small isolated populations.

Duplication. A type of chromosomal aberration in which part of a chromosome is duplicated.

Dysmorphism. Morphological developmental abnormality, as seen in many syndromes of genetic or environmental origin.

Empiric risk. Computation of probability that a given trait will occur or recur in a family based on past experience rather than based on knowledge of the causative mechanism.

Endogamy, Inbreeding. Mating within the group.

Epistasis. Interaction between products of two genes situated at different loci, where one gene masks or prevents the expression of another gene at a different locus. (Dominance is the term used when one gene masks one of its own alleles, i.e., another gene at the same locus).

Euchromatin. That chromatin which shows the staining behaviour characteristic of the majority of the chromosomal complement. It is uncoiled during interphase and condenses during mitosis, reaching a maximum density at metaphase.

Eukaryote. An organism in which the cells have a nucleus with a nuclear membrane.

Euploid. It is a polyploid chromosomal number that is an exact multiple of the haploid number. It can be normal ($2N$) or abnormal ($3N$, $4N$....).

Exons (Extrons). Parts of a gene (cistron) involved in protein synthesis by transcribing active mRNA.

Expressivity. The extent to which a gene is expressed in individuals. A trait with varying expressivity shows variation in degree of expression from mild to severe.

F. See coefficient of inbreeding.

Family. Parents and children. See also Kindred.

F_1, "F one", First filial generation. The first generation progeny of a mating, 'P', which stands for parental generation.

Fetoscopy. A procedure for direct visualization of the foetus used for prenatal diagnosis.

Fingerprint. The pattern of the ridged skin on the dis tal phalanx of a finger.

Fingerprint technique. A method of combining electrophoresis and chromatography to separate the components of a protein such as haemoglobin.

First-degree relatives. Closest relatives, i.e., parents, offspring and sibs.

Fitness (Darwinian, biological). The relative ability of an organism to survive and transmit its genes to the next generation. It is determined by the number of offspring who reach reproductive age. Fitness is unity (or 100 per cent) if a person has at least two such offspring.

Forme fruste. Any extremely mild expression of a genetic trait in an abnormality, disease or syndrome which is of no clinical significance.

G bands. The dark and light cross-bands formed on chromosomes after treatment with trypsin and Giemsa stain.

Gamete. A haploid germ cell (sperm or ovum).

Gene. A segment of a DNA molecule coded for the synthesis of a polypeptide.

Gene(s) in common. Genes which are inherited by two individuals from a common ancestral source.

Gene flow. Gradual diffusion of genes from one population to another by migration and miscegenation (interracial breeding).

Gene map. A representation of the human karyotype showing the locations of the genes on the chromosomes.

Gene pool. All the genes present at a given locus in a given population.

Genetic code. The base triplets of DNA or mRNA that specify the different amino acids.

Genetic counselling. Providing guidance to the problems related to the occurrence, or the risk of occurrence of a genetic disorder in a family. It also deals with the problems of the risk and the burden of the disorder and the options available for handling them.

Genetic death. Death of an individual without reproducing.

Genetic drift. The random fluctuations of gene frequencies due to sampling errors. While drift occurs in all populations, its effects are most evident in very small populations.

Genetic lethal. A genetically determined trait in which affected individuals do not reproduce.

Genetic load. The average number of lethal equivalents per individual in a population.

Genetic marker. A trait can be used as a genetic marker in studies of cell lines, individuals, families and populations, if (a) it is genetically determined, (b) can be accurately classified, (c) has a simple unequivocal pattern of inheritance and (d) has heritable variations common enough to allow it to be classified as a genetic polymorphism.

Genetic screening. Examining a population to detect individuals at risk of having a specific genetic disorder or of having a child with a specific genetic disorder. Genetic screening tests are normally applied only when some method of treatment or intervention is available.

Genetic trait. Trait determined by genes, not necessarily congenital. It may be manifested any time during life.

Genetics. The scientific study of heredity.

Genocopy, Gene mimic, Genetic mimic. See heterogeneity.

Genome. The full set of genes in a gamete or in an individual.

Genotype. The total genetic constitution (genome) of an individual, or more specifically the alleles present at one locus, for a particular trait.

Germ line. The cell line that produces gametes.

Gonosome. Sex chromosome, X or Y.

Haploid. The single set of all chromosomes, found in normal gametes. (Symbol: 1*N*).

Haplotype. A set of genes from closely linked loci on one chromosome, usually inherited as a unit. e.g. The four gene–loci of the HLA complex on chromosome 6 are very closely linked loci constituting a haplotype, thus each individual has two haplotypes. Within a kindred, the combinations of alleles at these loci are transmitted as units.

Hardy-Weinberg law. If two alleles (A and a) occur in a randomly mating population with the frequency of p and q, respectively, where $p + q = 1$, then the expected proportions of the three genotypes $AA = p^2$, $Aa = 2pq$, and $aa = q^2$, remain constant from generation to generation in an infinitely large, inbreeding population. Mutation, selection, migration, and genetic drift can and do disturb the Hardy-Weinberg equilibrium.

Hemizygous (hemizygotic). The genotype of a male with regard to an X-linked trait, since males have only one set of X-linked genes.

Hereditary, Heritable, Heredofamilial. Essentially synonymous terms for genetic traits transmissible through parents.

Heritability. A statistical measure of the degree to which a trait is genetically determined.

Heterochromatin. Chromosomal material staining differently from the rest of the chromosomal material in the cell during interphase, e.g., the Barr body. Also referred to as heterochromosome.

Heterochromosome. See *heterochromatin.*

Heterogametic sex. The sex that produces equal numbers of two different gametes. In man, it is the heterogametic male that produces X- and Y-bearing sperms. In birds, the female is heterogametic producing X- and Y-bearing ova.

Heterogeneity. Process in which an identical or similar phenotype is produced by a different genetic mechanism. Genocopy and genetic mimic are terms for a genetic trait which is phenotypically similar to, but fundamentally (genetically) distinct from another.

Heteroploid. Any chromosome number other than the normal.

Heterozygote, Heterozygous. An individual who possesses two different alleles at one particular locus on homologous chromosomes.

Histocompatibility. Antigenic similarity between the graft and the host. A graft is accepted by the host only if there is histocompatibility, i.e., graft has no antigens which the host does not have.

Histones. Basic protein core around which DNA coils in the chromosome. They are of four types, H_1, H_2A or H_2B, H_3 and H_4, rich in basic amino acids (lysine or arginine); and are found throughout the eukaryote evolution.

Holandric inheritance. The pattern of inheritance of genes on the Y chromosome. Only males are affected and the trait is transmitted by affected males to all their sons but to none of their daughters.

Homogametic sex. The sex that produces one type of gamete.

Homograft. See allograft.

Homologous chromosomes. Chromosomes that pair during meiosis. Each homologue is a duplicate of one of the chromosomes contributed at syngamy by the mother or the father. Homologous chromosomes contain the same linear sequence of genes and as a consequence, genes as a rule occur in pairs.

Homozygote, Homozygous. An individual who possesses two identical alleles at one particular locus on homologous chromosomes.

Hybrid. The progeny of a cross between two genetically different organisms.

Iatrogenic, Iatral. Literally, caused by a physician. The term refers to any condition which results from medical treatment.

Immune competent. Cells capable of producing an antibody in response to an antigenic stimulus are immune competent.

Immune reaction. The specific reaction between antigen and antibody.

Immunogenetics. Studies using a combination of immunologic and genetic techniques, as in the investigation of genetic characters detectable only by immune reactions.

Immunoglobulin. See antibody.

Immunological homeostasis. The characteristic condition of a normal adult, who has certain antigens and the ability to react to antigens by producing antibodies, but who does not produce antibodies to his own antigens.

Immunological tolerance. The inability to respond to a specific antigen because of previous exposure to that antigen, especially during embryonic life.

Inborn error. A genetically determined biochemical disorder in which a specific enzyme defect produces a metabolic block which may have pathological consequences.

Inbreeding. The mating of closely related individuals. The progeny of close relatives are said to be inbred. In laboratory mice, brother-sister inbreeding over many generations has been used to produce inbred lines.

Incompatibility. The donor and host are incompatible if, because of a genetic difference, the host rejects cells transplanted from the donor. In maternal-foetal incompatibility, the mother forms antibodies against foetal cells which have entered her circulation.

Insertion. A structural abnormality of chromosome in which a part of an arm of one chromosome is inserted into the arm of a nonhomologous chromosome.

Interchange. See translocation.

Introns. Inserts or parts of a gene, alternating with exons, and with them, concerned in transcribing heterogeneous nuclear RNA (hnRNA or precursor RNA).

Interphase. The major portion of the cell cycle, during which a cell is not undergoing division. The stage between two successive mitotic divisions during which DNA replication occurs.

Inversion. A type of chromosomal aberration in which part of a chromosome is reversed end-to-end. When occurring in one of the arms of a chromosome, the inversion is termed paracentric; when the inverted segment includes the centromere, it is called pericentric.

Isoalleles. Allelic genes which are "normal" and can be distinguished from one another only by their differing phenotypic expression when in combination with a dominant mutant allele.

Isochromosome. A type of chromosomal aberration in which one of the arms of a particular chromosome is duplicated because the centromere divides transversely and not longitudinally during cell division. The two arms of an isochromosome are therefore of equal length and contain identical genes. This is an example of duplication, and deletion.

Isograft. A tissue graft between two individuals who have identical genotypes, as between MZ twins.

Isolate. A segment of a population within which assortative mating occurs.

Karyotype. The chromosome set of a somatic cell. A photomicrograph of an individual's chromosomes arranged in a standard fashion.

Kindred. An extended family group where each one is related, genetically or by marriage, to every other member of the group.

Kinetochore. Centromere; primary constriction of a chromosome.

Lethal equivalent. It is one gene which, if homozygous, would be lethal, or two genes which, if homozygous, would be lethal in half the homozygotes and so on.

Linkage. Greater association in inheritance of two or more non-allelic genes, than is to be expected from independent assortment, because of location of the genes on the same chromosome.

Locus. The position or site of a gene on a chromosome. Different alleles are always found at the same position on the chromosome. A complex locus is a group of loci within which, at a time, mutation and recombination can occur at more than one site.

Lyon hypothesis, Lyonization. The hypothesis advanced by Mary F. Lyon that in mammals, one or the other of the two X-chromosomes of the female is inactivated in embryonic cells and their descendants; and that mammalian females are consequently X-chromosome mosaics. See Barr body, sex chromatin.

Manifesting heterozygote. A female heterozygous for an X-linked disorder, in whom, because of Lyonization of the "normal" X-chromosome the trait is expressed clinically with approximately the same degree of severity as in hemizygous affected males.

Map unit, Map distance. The measure of distance separating loci on a chromosome as inferred from the recombination fraction.

Mapping a chromosome. Determining the position and order of gene loci on a chromosome, especially by analyzing the frequency of recombination between the loci.

Meiosis. The special type of cell division occurring in the germ cells by which gametes having the haploid chromosome number are produced from diploid cells. Two meiotic divisions occur, first and second (meiosis I and meiosis II). Reduction in number takes place during meiosis I, and further reduction of genetic material during meiosis II.

Mendelization. The attribute of traits which show simple qualitative patterns of inheritance.

Messenger RNA. The RNA which has a base sequence complementary to a DNA strand, and is formed during "transcription" to function during "translation" as a template for synthesis of a polypeptide.

Metacentric. Designating a chromosome with a nearly centrally placed centromere. Also called 'median'.

Metaphase. The stage of a cell division when the contracted chromosomes, each consisting of two chromatids, line up on the equatorial plate following the disappearance of the nuclear membrane.

MHC. Abbreviation for the major histocompatibility complex, H-2 in the mouse or HLA in humans.

Mimic genes. Different genes with similar phenotypic effects. Also called gene mimics or genocopies.

Mitosis. Somatic cell division resulting in the formation of two daughter cells, each with the same chromosome complement as the parent cell.

Mitotic cycle. The cycle of a cell between two successive mitoses, in which four periods are distinguished: G_1 (Gap 1), S (DNA Synthesis), G_2 (Gap 2) and M (Mitosis).

Monosomy. The state where only one member of a given homologous chromosomal pair is present, e.g., the single X of XO, as in Turner's syndrome. Partial monosomy results when part of a chromosome is deleted so that the portion of the homologous chromosome is monosomic.

Monozygotic, Identical, Monozygous. Twins derived from a single fertilized ovum. Such twins are genetically identical.

Mosaic, Mixoploid. An individual composed of cells of different genotype or karyotype derived from a single zygote. Mosaicism is brought about by gene or chromosomal mutation in somatic cells. It does not include different cell types arising by neoplasia or chimerism. Gonadal mosaicism results from a somatic mutation early in embryogenesis so that some or all the germ cells are of a mutant type. A person with a gonadal mosaicism for a dominant trait can have two or more children with that trait without showing it himself. Gonadal mosaicism may also involve chromosomal aberration.

Multifactorial. A trait determined by multiple factors, genetic and nongenetic (environmental), each with only a minor additive effect.

Multiple alleles. The existence of more than two different alleles at a given locus of a chromosome in a population. In a given individual only two of these alleles occur, one derived from each parent.

Mutant. An individual bearing a gene in which a mutation has occurred, that expresses itself in the phenotype of the organism.

Mutation. 1. Process by which a gene or chromosome undergoes a structural change. 2. Modified gene resulting from mutation. 3. By extension, the individual manifesting the mutation, i.e., a mutant. The term is commonly used to describe a change in a single gene-locus (point mutation). A mutation which occurs in the somatic cells (somatic mutation) cannot be transmitted to the next generation.

Mutation rate. The number of mutations of any one particular locus which occur per gamete per generation.

Muton. The smallest unit of DNA which can be involved in a mutation. Since change in a single base pair changes the genetic code, muton is equivalent to one base pair.

Non-disjunction. The failure of separation of paired chromosomes or of chromatids during cell division, resulting in one daughter cell receiving none of the chromosomes in question. Non-disjunction can occur during a meiotic or mitotic division.

Non-penetrance. Failure of a genetic trait to be evident in a population even though the genotype usually productive of that phenotype, is present. Opposite of expressivity.

Nonsecretor. See secretor.

Nucleoside. A purine or pyrimidine base attached to a 5-carbon sugar (deoxyribose or ribose).

Nucleosome. A globular, repetitive constituent of chromatin fibre, consisting of a histone core, around which is wound a variable length of DNA (about 140 base pairs).

Nucleotide. A nucleoside attached to a phosphate group. A nucleic acid molecule is a polymer of many nucleotide units.

Oogenesis. The process of formation of the female gametes, from primordial germ cells to mature ovum.

Operator gene. A gene which switches on adjacent structural gene (s).

Operon. A postulated unit of gene action, consisting of an operator gene and the closely linked structural gene(s), whose action it controls.

Optimon. A unit of natural selection. Also called *selecton.*

p. 1. The short arm of a chromosome (from the French petit=small). 2. Often used to indicate the frequency of the more common allele of a pair, in the statement of Hardy-Weinberg law.

Panmixis. Random mating.

Pedigree. A diagrammatic representation of family history, indicating the affected and normal individuals, gene carriers and their relationship to the propositus.

Penetrance. It is the frequency of expression of a genotype in a population. When the frequency is less than 100 per cent, the trait is said to exhibit reduced penetrance. See non-penetrance.

Pharmacogenetics. The area of biochemical genetics concerned with responses to drugs and their genetically controlled variations.

Phenocopy. The alteration of the phenotype, by nutrition or the exposure to environmental stress during development, to a form imitating that characteristically produced by a specific gene. Thus rickets due to a dietary lack of Vitamin D would be a phenocopy of vitamin D-resistant rickets. A sun tan is a phenocopy of the genetically determined brown skin.

Phenodeviant. A member of a population different significantly in phenotype from the population as a whole.

Phenotype. The appearance (physical, biochemical, and physiological) of an individual, produced by the interaction of environment and the genotype.

Philadelphia chromosome. An aberrant human chromosome. Ph^1 is found in patients suffering from chronic myeloid leukaemia. The chromosome is believed to be chromosome 21 or 22 minus a substantial portion of its long arm (22q minus).

Plasmagenes. Genes situated within the cytoplasm of the cell.

Pleiotropy, Polypheny. The phenomenon of a single gene being responsible for a number of distinct and seemingly unrelated phenotypic effects.

Point mutation. See mutation.

Polygenic, Quantitative. Determined by many genes at different loci, with small additive effects. To be distinguished from multifactorial, in which environmental as well as genetic factors are involved. However, to many a geneticist, polygenic and multifactorial are synonymous.

Polymorphism. The occurrence together, in the same habitat, of two or more discontinuous forms of a trait, in such proportions that the rarest of them cannot be maintained in the population by mutation alone. The existence of two or more genetically different classes in the same interbreeding population, e.g., Rh-positive and Rh-negative humans. The polymorphism may be transient, or the proportions of the different classes may remain the same for many generations. In the latter case, the phenomenon is referred to as balanced polymorphism. Geographic polymorphism is said to exist when the classes are located in different regions. Over forty genetic polymorphisms in serum proteins, red cell antigens and red cell enzymes are known.

Polypeptide. A chain of one or more peptides. Each peptide has two or more amino acids, held together by peptide bonds between the amino group of one and the carboxyl group of the adjoining one. A protein molecule may be composed of a single polypeptide, or of two or more identical or different polypeptides.

Polyploid. Any multiple of the basic haploid chromosome number, other than the diploid, thus 3*N* 4*N* and so on.

Polysome, Polyribosome. A group of ribosomes associated with the same molecule of messenger RNA.

Polyteny. A multi-stranded chromosome in which, following replication, the sister chromatids do not separate.

Prenatal diagnosis. Determination of the sex, karyotype or phenotype of a foetus, usually prior to the 20th week of gestation. A variety of techniques, especially amniocentesis and cell culture, is employed.

Primary constriction. See centromere.

Proband, Index case. An affected individual (irrespective of sex) through whom the family came to the attention of the investigator is called propositus, if a male; proposita, if a female; (plurals: propositi and propositae.)

Prophase. The first stage of cell division, during which the chromosomes become visible as discrete structures and subsequently thicken and shorten. Prophase of the first meiotic division is further characterized by pairing (synapsis) of homologous chromosomes.

Propositus, Proposita. See Proband.

Pseudoalleles. Genes at two closely linked loci concerned with the same function; rarely can be separated by crossing over. Though known in experimental organisms, they have not been demonstrated in man.

q. 1. The long arm of a chromosome. 2. Often used to indicate the frequency of the rarer allele of a pair, as in the statement of the Hardy-Weinberg law.

Q bands. The pattern of bright and dim cross-bands seen on chromosomes under fluorescent light after quinacrine staining.

Quasicontinuous variation. The type of variation shown by a multifactorial trait which becomes manifest only on crossing a certain threshold and thus appears to have a discontinuous distribution.

Quasidominant, Quasidominance. The direct transmission, from generation to generation of a recessive trait. This pattern of inheritance is produced by the mating of a recessive homozygote with a heterozygote, so that recessively affected members appear in two successive generations and the frequency of affected persons in the second generation is 1/2 that of the first generation.

hnRNA (Precursor RNA). It is the initial, faithful transcript of a gene, containing 'active' and 'inactive' portions.

Random genetic drift. See Drift.

Random mating, Panmixis. Selection of a mate without regard to the genotype of the mate. In a randomly mating population, the frequencies of the various matings are determined solely by the frequencies of the genes concerned.

Recessive. A trait which is expressed in homozygotes for a particular gene but not in those who are heterozygous for that gene.

Recombinant. An individual who exhibits a recombination of genes.

Recombinant DNA. Artificially synthesized DNA in which a gene or part of a gene from one organism is inserted into the genome of another.

Recombination. The occurrence of progeny with combinations of genes other than those that occurred in the parents, due to independent assortment or crossing over.

Recon. The smallest unit of DNA capable of recombination, crossing over can occur within the cistron; so that the smallest unit of recombination may be any two successive nucleotides in a codon.

Recurrence risk. Is the probability that a genetic disorder, present in one or more members of a family, will recur in another member of the same or a subsequent generation.

Reduction division. The first meiotic division, where the chromosome number is reduced from diploid to haploid per cell. Also see meiosis.

Regulator gene. A gene whose primary function is to control the rate of synthesis of the products of other genes. The regulator gene (rG) controls the synthesis of a protein repressor (R), which inhibits the action of an operator gene (oG) and thus turns off the operon it controls.

Repulsion. In a double heterozygote, linkage is said to be in the repulsion phase when the mutant alleles of interest at the two loci are on opposite chromosomes.

Ring chromosome. A structurally abnormal chromosome, in which the ends of both arms, break off and get deleted and the remainder of the chromosome becomes a ring by the mutual reunion of its ends.

RNA, Ribonucleic acid. Any of a family of polynucleotides characterized by their component sugar (ribose) and one of their pyrimidines (uracil). RNA molecules are single stranded and have molecular weights lower than that of DNAs. There are four classes of RNAs: messenger RNA, ribosomal RNA, transfer RNA and viral RNA.

Robertsonian translocation. See centric fusion.

Secretor. The person who secretes ABO blood group substance into saliva and other body fluids.

Segregation. The separation of allelic genes at meiosis, i.e., during gametogenesis. Since allelic genes occupy the same locus on homologous chromosomes, they pass to different gametes, i.e., they segregate.

Selection. 1. The manner in which kindreds are chosen for study, i.e., ascertainment. 2. In population genetics, the operation of forces that determines the relative fitness of a genotype in the population.

Selecton. See Optimon.

Sex chromatin. See Barr body.

Sex chromosomes. Chromosomes responsible for sex determination. (In man: XX in females, XY in males).

Sex-influenced, Sex-conditioned. A trait which is not X-linked in its pattern of inheritance but is expressed differently (either in degree or in frequency) in males and females, e.g., a sex-influenced, autosomal gene may behave as a dominant in males and as a recessive in females. Furthermore in the homozygous female the condition may be expressed to a minor degree, e.g., pattern baldness.

Sex-limited. A trait which is expressed in only one sex, though the gene determining it is not X-linked.

Sex-linkage. Genes carried on the sex chromosomes. Since there are very few Mendelizing genes on the Y chromosome, the term is often used synonymously for X-linkage.

Sex-ratio. The relative proportion of males and females of specific age distribution in a population. The primary sex ratio refers to that at fertilization: the secondary sex ratio refers to that at birth.

Sib, Sibling. Brother or sister.

Sibship. Group of brothers and/or sisters.

Silent allele. An allele which has no detectable product.

Sister chromatid exchange. Exchange of DNA segments between sister chromatids, during mitosis. This occurs very frequently in patients with Bloom's syndrome.

Somatic cell genetics: The study of genetic phenomena in cultured somatic cells.

Somatic mutation. See mutation.

Spermatogenesis. The process of formation of spermatozoa. It includes both meiosis and spermiogenesis.

Spermiogenesis. The formation of sperm from the spermatids produced during the meiotic divisions of spermatocytes.

Spindle. Microtubular complex seen in the cell during cell division. It extends from centriole to the centromeres of chromosomes. It enables the arrangement of chromosomes on equator at metaphase and their segregation at anaphase.

Sporadic. A trait, occurring in an isolated case within a kindred, which has no known genetic basis.

Structural gene. See cistron.

Syndrome. A group of symptoms and signs that occur together, characterizing a disease.

Synteny, Syntenic. Presence together on the same chromosome of two or more gene-loci, whether or not they are close enough together for linkage to be demonstrated.

T cells. Small lymphocytes (thymus-dependent cells) responsible for cell-mediated response to antigens.

Telocentric. Refers to chromosome with a terminal centromere.

Telophase. The stage of cell division which begins when the daughter chromosomes reach the poles of the dividing cell and lasts until the two daughter cells take on the appearance of interphase cells.

Teratogen. Any agent that produces or raises the incidence of, congenital malformations.

Tetraploid. Having four haploid sets of chromosomes in the nucleus.

Trait. Any gene-determined characteristic. Although in medicine it has come to be used particularly for the heterozygous state of a recessive disorder such as sickle-cell anaemia, it has a more general meaning in genetics.

Transcription. The formation of messenger RNA against a DNA template.

Transduction. Change in the genetic constitution of an organism (e.g., bacterium) by treatment with DNA from a different strain. (A bacteriophage is used as a vector.)

Transformation. A form of recombination of genetic material in bacteria in which a bacterium incorporates DNA extracted from other bacteria into its own genetic material.

Translation. The formation of a protein directed by a specific messenger RNA molecule.

Translocation. A chromosomal aberration involving transfer of a piece of one chromosome to a nonhomologous chromosome. If two nonhomologous chromosomes exchange pieces, the translocation is reciprocal. When an individual or gamete carries neither more nor less than the normal diploid or haploid genetic material, respectively, the situation is referred to as balanced translocation. See also centric fusion.

Triplet. A unit of three linear successive bases in DNA or RNA which codes for a specific amino acid.

Triploid. A cell having three haploid sets of chromosomes, or an individual made up of such cells.

Triradius. In dermatoglyphics, a point from which the dermal ridges course in three directions at angles of approximately 120 degrees.

Trisomy. The state that is diploid but contains one extra chromosome, homologous with one of the existing pairs, so that one kind of chromosome is present in triplicate e.g., trisomy 21 (Down's syndrome).

Ultrasonography. A procedure to delineate the outline of various structures using high frequency sound waves. Often used in prenatal diagnosis.

Unifactorial, Mendelizing. Inheritance controlled by a single gene pair.

Wild type. Term used especially in experimental genetics to indicate most frequently observed phenotype, or one arbitrarily designated as "normal".

Wild type gene. The allele commonly found in nature or arbitrarily designated as "normal" (often symbolized as +) or normal homozygote (+/+).

X-inactivation. See Lyon hypothesis.

X-linkage. Genes carried on the X chromosome are said to be X-linked.

Xenograft. Graft from a donor of one species to a host of a different species.

Zygote. The diploid cell resulting from the union of the haploid male and female gametes. The term is also used to refer to the organism that develops from the zygote.

Zygosity. The term refers to multiple births in relation to their origin vis-a-vis the zygote. e.g., Twins may be either monozygotic or dizygotic. To determine which type a certain twin pair represents is to determine the zygosity of the pair.

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INDEX

N.B. Italicized page numbers refer to illustrations and tables

ABO blood groups, 2, 92, 111, *118*
Acatalasia, Acatalasemia, *101*, 108
Acetylsalicylic acid, 109
Achondroplasia, 84
Acquired tolerance, 115
Acrosome, *25*, 26
Activator, 66
Adenine *51*, 51, 53
ADP, 51
Agammaglobulinaemia, *112*, 117
Albinism, 2, *85*, 86, 100, *101*
Alkaptonuria, 2, *85*, 100
Alleles, 1, 16, 82
  multiple, *see* Multiple alleles
Allelomorphs, *see* Alleles
Allograft, 115
Allograft reaction, 115
Allotypes, 112
Amniocentesis, 138, 141
Amniography, 138, 141
Amorph, 119
AMP, 52
Anaesthetic agents, 108, 109
Anaphase
  in meiosis, *21*, 22
  in mitosis, *18*, *19*, 20
Anencephaly, 139
Aneuploidy 6, 42
Annealing, 93, *148*
Antenatal diagnosis, 138, 141
Antibody, 100, 111
Anticipation, 95
Anticoagulants, 108, 109
Anticodon, 59
Antigen, 111
Apert's acrocephalosyndactyly, 70
Arber, 3
Argininaemia, 140
Ascopore, 144
Association, 91, 98
Assortative mating, 127
Asters, 20
Ataxia telangiectasia, 117
ATP, 51
Atretic follicles, 30
Autograft, 115
Autoimmune diseases, 111, 114, 115, 117
Autoimmunity, 71, 111, 115
Autoradiography, 93, 148
Autosome/s, 5, 7
Autosomal linkage, 2
Avery-Macleod-McCarty experiment, 145
Axoneme, 26

Bacteriophage, 67, *145*, 145
  reproduction, *145*, 146
Baltimore, 3
Banding, 5, 10, *11*, 12
  AgNOR staining, 12
  C bands, 12, 58
  Cd, 12
  G bands, 10, *11*, 12
  G-11 staining, 12
  Hoechst 33258 banding, 12
  pattern, 8, *94*
  Q banding, 10, *11*, 12
  R banding, *11*, 12
  special techniques, 12
Barbiturates, 109
Barr, 5, 13
Barr body, 13, *14*, 30, 88, *see also* Sex chromatin
Base substitution, 71
Beadle, 2, 100
Bertram, 5, 13
Blood groups, *85*, 87, 93, 96, 100, 117, *119*, *120*, 125
  ABO, 2, 92, 111, *118*, 118
    Bombay phenotype, 119
    isoagglutination, 118
    secretion, 120
  Diego, 123
  Dombrock, 124
  Duffy, 75, 123
  I, 123
  Kell, 123
  Kidd, 123
  Lewis, *123*, 123
    secretors and nonsecretors, *123*, 123
  Lutheran, 2, 92, 122
  MNSs, *85*, *122*, 122
  P, 122
  notations, 118
  Rh, 111, *120*, 120
    -D-/-D-/-D-D-, 120
    Fisher-Rice notation, *120*, 120
    incompatibility, 122, 125
    Rh null, 120
    Wiener notation, *120*, 120
  Xg, 49, 89, 92, 93, *124*, 124
  Yt (Cartwright), 124
Blood transfusion, 111, 125
Brachydactyly, 84
Bruton-type agammaglobulinaemia, *112*, 117
Bursa of Fabricius, 115

C bands, 12, 58
Cancerogenesis, 69
Capping, 61, 68
Carrier, 70, *83*
  detection of, 137
Caspersson, 10
Cell cycle, *18*, 19
Cells of Leydig, 23, *24*
Cells of Sertoli, 23, *24*
Centimorgan, 93
Centriole, *25*, 26
Centromere, 5, 7, *7*, 58 *see also* Constriction, primary
Chain termination, 64
Character, 1
Chiasma, 22
Chimera, 115, 124, 133
Chimerism, 130
Chlorthiazide, 109
Chromatid, 7, *7*, 35
Chromatin, 6, *17*
  body, *see* Barr body
  eu–,6
  fibre, 18
  hetero–,6
  sex, 13, *14*, 30 *see also* Barr body
  structure, 65
Chromosomal aberrations, 5, 32, *45*, 49, 133 *see also* Chromosome, abnormal
  carrier of, 32
  clinical syndromes due to, 43
  deficiency, 33
  deletion, 32, *33*, 33, 39, 49, 74
  duplication, 32, 40, 49
  insertion, *38*, 38, 49
  in number, 41, 49
  inversion, 32, 39, 49
    paracentric, 39, 49
    pericentric, 39, 49
  isochromosome, 38, 49
  mapping, 93
  non-disjunction, *23*, 41, 49, 55, 71
  ring, 32, *34*, 34, 35, 49
  structural, 32, 49
  translocation, 32, 34, 49
    reciprocal, 34, *36*
    Robertsonian (centric fusion), 36, *37*
    simple, 36
Chromosomal behaviour in cell division, 18
Chromosomal manoeuvres, 18
Chromosomal mapping, 92, 142, 143
Chromosomal nomenclature, 9
Chromosomal polymorphism, 13

Chromosome/s, 5, *7, 9, 10, 11, 17*, 30
  abnormal
    causes of, 32, 48, 49
  acrocentric 7, 8, *10*, 13, *37*, 55, 71
  analysis, 9
    uses of, 12
  autosomes, 5, 7
  banding, 10, *11, 12*
  classification, 5, 30
    basis, 5
    Denver, 8
    Denver-London, 8
    Paris nomenclature, 8, *11*
  composition of, 16
  crossing over 22, 34, *35*, 91, 143
    unequal, *40*, 40, *41, 42*
  DNA in, *see* DNA
  functions of, 16
  homologous, 6, *17, 35*, 74, 81
  identification, 10
  long arm, 7, 8, 9
  mapping, 92, 93, *94*, 142
  metacentric, 8
  methods of study, 10, 30
  nucleolar, 8
  number
    aneuploidy, 42, 49
    diploid, 41, 50
    euploid, 41, 49
    haploid, 41, 50
    heteroploid, 42
    polyploid, 49
    tetraploid, 41
    triploid, 41
  Philadelphia, 12, 37
  preparation for study, 9
  SAT-7, 8
  sex, 7, 13, *45*
    X, 2, 5, 6, 7
      inactivation of, 16
    XO, 13, 15, *see* Turner's syndrome
    XXX, 43, 46
    XXY, *see* Klinefelter's syndrome
    XYY, 43, 48
  short arm, 7, 8, 9
  structure of, 7, 16, 30
  study of, 30
  submetacentric, 8, *10*, 13
  telocentric, 7, 8
  theory of inheritance, 2
Chronic myelogenous leukemia (CML), 12, 37
Cistron, 59, *60*, 69
Cleft lip, 137
Cleft palate, 95, 137
Codominance, *85*, 98, 111
Codon, 53, 67, 79
Colchicine, 9
Colinearity of genes, 68
Colour blindness, 2, 87, *88*, 93
  partial, 87, 88, 92
Complement, 114
Compound, 82
Congenital adrenal hyperplasia, *95*, 141
Congenital hypothyroidism, 141
Congenital malformations, 78, 133
Consanguineous marriages, 2, 70, 136
Consanguineous mating, 127
Constrictions,
  primary, 5, 7
  secondary 8, 55
Corepressor, 60
Cori, Carl & Gerty, 2
Corona radiata, *29*, 30
Corpus luteum, *27*, 28
Correns, 1
Cortex, of ovary, *27*, 27
cri du chat syndrome, 33, 43, 45
Crossing over, 22
  abnormal, 72
  between chromosomes, 91
  unequal, 72
Cross over, *see* Crossing over
Cumulus oophorus, *29*, 30
Cyclic AMP, 61
Cystathionine synthetase, 77
Cystic fibrosis, 88, 141
Cystinuria, 100, *101*
Cytogenetics, 3, 5, *see also* Chromosomes
Cytosine, *51*, 51, 53

Darwinian theory of biological fitness, 128
D trisomy, 43, 45
Degenerate code, *see* Genetic code
Dentinogenesis imperfecta, 84
Deoxyribonucleic acid, *see* DNA
Dermal ridge patterns, 126
Dermatoglyphics, 44, *45*, 129, *130, 131, 132*, 133
de Vries, 1
Diabetes mellitus, *134*, 137
Diakinesis, 22
Diaphragmatic hernia, 138
Differentiation, 69
Dinucleotide, 51
Diploid, 5, 6, 41, 50
Diplotene, 22
Dispermy, 128
Disputed paternity, 122, 137
Division, cell
  chromosomal behaviour in, 18
  meiosis, 20, 31
    meiosis I, 21, 28, 31
    meiosis II, 21, 28, 31
  mitosis, 19, 30
DNA, *16*, 50, 51, *53*, 53, *54, 57, 63*, 79
  as genetic material, 6
  base sequence of, 67
  classification of, 55
  complementary, 77
  mitochondrial, 53
  modification, 65
  organisation in chromosome, 16
  polymerase, 76
  polymorphisms, 69
  rearrangement, 65
  redundant, 69
  repair, 75, *76*
  replication of, 54, *56*, 57
    semiconservative, 54
  satellite, 57, 58
  sequencing, 61, 79, 149
  structure of, studies on, 3, 53, 79, 146
Dominant, 70, 82
Dominant inheritance, *see* Inheritance, dominant
Down's syndrome, 3, 32, 36, 39, 43. *44, 45*, 129, 136, 137
  clinical features, 44
  cytogenetics, 44
  dermatoglyphics, 44, *45, 131, 132*
Drosophila melanogaster, 33, 66. 69, 78, 126, 142, *143, 144*, 149
Drumsticks, 14
Duchenne muscular dystrophy, 70, 80, *89*, 93, 96, *134*, 139, 141
Ductus deferens, 23
Duplications, *see* Chromosomes
Dysgammaglobulinaemias, 117

E syndrome, 45
Ears, hairy, 87
Ecdysone, 78
Effectors, 60
Efferent ductules, 23
Ejaculatory ducts, 23
Embryogenesis, 77
Embryonic differentiation, 69
Embryonic induction, 78
Empiric risk, 137
Endonuclease enzyme, 76
Environment and heredity, 1, 4
Epididymis, 23
Erythroblastosis fetalis, 122, 125
Euchromatin, 6
Eugenics, 2
Eukaryotes, 5
Euploid, 41
Exons, 61, *63*, 65, 79
Exonuclease enzyme, 76

Expressivity, 90, 98

Favism, 109
Fertilization, 6
Fetography, 138
Fingerprints, 129
Fitness, Darwinian, 128
Fluctuating asymmetry, 132
Follicles, atretic, *27*, 30
  Graafian, *27*, *29*, 29
  primary, *27*, 28
  secondary, 28
  vesicular, 29
Ford, 3
Formaldehyde, 75, 79
Forme fruste, 90, 98

Galactosemia, 72, 86, 100, *101*, *134*
Galton, 2, 4, 128
Gamma rays, 75
Gametes, 1, 6, 23
Gametogenesis, 1, *37*
Garden peas, 1, 4, 142
Garrod, 2, 4, 100
Gastrointestinal atresias, 138
Gaucher's disease, 86, *101*
Gaussian curve, 96, *97*
Genes, 5, 79, *see also* Alleles
  colinearity of, 68
  differential activity of, 77
  dominant, 81
  duplication, 72
  flow of, 128
  heterozygous, 81
  hexon, 69
  homologous, 81, 82
  jumping, 3, 66
  libraries, 77, 142
  locus of, 5, 6
  marker, 13, 93
  mitochondrial, 50
  operator, 59, 61
  overlapping, 66, 67
  promoter, 60, 61
  recessive, 81
  regulator, 59, *60*
  split, 67
  structural, 55, 57, 59, *60*, 61
  structure of, 61
  synthesis of, experiments for, 147
  turning on or off, of, 69
  variation in expression of, 90, 98
  X-linked, 15
Gene action, protein structure and, 64
Genetic code, 53, *55*, 79
  cracking of, 3, 146
  degenerate, 54
Genetic control of protein synthesis, 3
Genetic counselling, 4, 134, 141
  carrier detection, 134, 137, *138*, 141
  disputed paternity, 134, 137, 141
  for child adoption, 134, 137, 141
  in consanguinity, 134, 136, 141
  risk of recurrence, 134, *135*,135, 141
Genetic drift, *see* Random genetic drift
Genetic heterogeneity, *see* Heterogeneity, genetic
Genetic mapping, 67
Genetics, 1, 4,
  applied aspects of, 1
  bean-bag, 126
  beginning, 1
  biochemical, 3, 4, 100, 109
  central dogma of, 3
  cyto, 3
  developmental, 4, 77, 79
  experimental, 4
  immuno, 4, 111, 124
  medical, 2
    applied, 4
  microbial, 144
  molecular, 3, 51, 79
  of haemoglobins, 104
  pharmaco, 100, 108, 110
  population, 2, 4, 126, 133
  quantitative, 2
  transplantation, 115, 124
Genetic trait, *see* Trait
Genital system,
  female, 27
  male, 23
Genome, 16, 50, 55, 67
Genonemata, 16
Genotype, 50, 81, 82
Giemsa method, 5
Germinal epithelium, *27*, 27
Glucose-6-phosphate dehydrogenase (G6PD)
  deficiency of 2, 75, 88, 93, *101*, 103, 109, 140
Glucuronyl transferase, 140
Golgi complex, 64
Gonad,
  female, *27*, 27
  male, 23, *24*
Gout, 109
Graafian follicle, *27*, *29*, 29
Graft, 115
Graft rejection, 114
Graft-versus-host reaction, 115
Griffith experiment, 145
Guanine, *51*, 51, *53*
Guthrie test, 103

Haemoglobin, 103, *105*, *106*, 109
  A, 104
  A2, 104
  abnormal, 71, 100, 105, *106*
  adult, 104
  Bart, 104, 106
  C, 71, 75, 106
    Georgetown, 107
    Harlem, 107
  Constant Spring, 107
  embryonic, 104
  F, 104
  foetal, 104
  Freiburg, 107
  Gower I, 104
  Gower II, 104
  Gun Hill, 107
  H, 104, 106
  Hopkins, 104
  Lepore, 41, 72, 106
  Portland, 104
  Sickle, 2, 71, 105, 107
  Zurich, 109
Haemoglobinopathies, 86, 101, 105, 109, 141
Haemolysis, 109
Haemolytic disease of the newborn, 122, 125
Haemophilia, 2, 87, 88, *89*, 93, 139
Hairy, pinna, 87
Haldane, 2
Halothane, 109
Hamerton, 3
Haploid, 5, 6, 41, 50
Haptoglobin, 41, 72, 93
Hardy, 2
Hardy-Weinberg principle, 2, 126, 133
  factors affecting, 127, 133
Harelip, 95
Hashimoto's thyroiditis, 39
Hemizygous, 87
Hepatolenticular degeneration, 86, 100
Hereditary angioneurotic oedema, 114
Hereditary spherocytosis, 140
Heredity, 2, 4
  mathematical aspect of, 2
Hermaphrodite, 43, *48*, 48
  female, 43, 48
  male, 43, 48
  pseudo, 43, 48
  true, 43, 48
Hermaphroditism, 48
Hershey-Chase experiment, 145
Heterochromatin, 6, 13
  constitutive, 6
  facultative, 6
Heterogametic sex, 7
Heterogeneity, genetic, 71, 81,

94, 98, 136
Heteroploid, 42
Heterozygous (heterozygote), 70, 81, 82
Hexon gene, 69
Histocompatibility, 116
Histocompatibility locus A, *116*, 116
Histones, *16*, 16, 54, 65, 78
HL-A system, 116, 124
Hogness box, 61
Holandric inheritance, *see* Y-linked inheritance
Holley, 3
Homogametic sex, 7
Homograft, 115
Homologous chromosomes, *see* Chromosomes
Homozygous (homozygotic), 81, 82
Hungerford, 3
Hunter's syndrome, 100, *102*
Huntington's chorea, 70, 137
Hurler's syndrome, 72, 100, *102*
H-Y antigen, 13
Hybrid, 112
Hybridization, somatic cell, 93, 142, *144*
  southern, 69, *147*
Hydatid mole, 139
Hydrogen peroxide, 108
Hyperacute response, 115
Hypokalaemic periodic paralysis, 70
Hypogammaglobulinaemias, 117

Idiopathic epilepsy, 137
Idiopathic scoliosis, 137
Immune competence, 115
Immune competent cells, 115
Immune reaction, 111
Immune response, 111, 112
  cellular, 111, 112
  humoral, 111, 112
Immunogenetics, 111, 124
Immunoglobulin, *see* Antibody
Immunoglobulins, *113*, 113
  Fab fragment, *113*, 113
  Fc fragment, *113*, 113
Immunological deficiency disease, 111, *112*, 117, 124
Immunological homeostasis, 111, 115
Immunological tolerance, 111
Inborn errors of metabolism, 2, 4, 72, 100, 109
Incompatibility, 112
Index case, 82
Inducers, *60*, 61, 78
Inheritance,
  autosomal, 81, *85*
  codominant, 81, 87, 98
  dominant, 81, 82, *84*, 84, 98, 135
    homozygous, 81
    intermediate, 81, 87, 98
    recessive, 81, 82, *86*, 86, 98, 103, 108, 135
  holandric, 81, 90, 98
  maternal, 53
  Mendelian, 81
  modes of, 81
  polygenic, 2, 81, 82, 96, 98, 108
  sex-linked, 81, 87, 98
  single gene trait, 82, 97
  unit, 81, 82
  X-linked, 82, 87, 98, 103, 135
    dominant, *89*, 89, 98
    recessive, *88*, 88, *89*, 98, 135
  Y-linked, 81, 90, 98
Initiation codon, 67
Initiation complex, 64
Inserts, 68
Interphase, *18, 19*
Intersex, 43, 48
  ascertainment of, 14
Interstitial cells of Leydig, 23, *24*
Introns, 61, *63*, 65, 68, 69, 79
Inversion, chromosomal, *see* Chromosomes
Isochromosome, 38
Isoenzymes, 78
Isograft, 115
Isoniazid, *101*, 108

Jacob-Monod model, 59
Jacob, 3
Jerne, 3
Jervis, 3

Karyotype, 5, 6, 10, 30
Karyotyping, 5
Khorana, 3, 147
Klinefelter's syndrome, 32, 43, 46, *47*, 137
Kohler, 3
Kornberg, 3

Lac operon, 61
Lactic dehydrogenase, 78
La Du, 3
Landsteiner, 3, 4
Lederberg, 3
Lepore-type mutants, 72
Leptotene, 21
Lejeune, 3
Lesch-Nyhan syndrome, 100, 103
Lethal equivalent, 75
Levan, 3
Leydig cells, 23, *24*
Linkage, 13, 91, *92*, 92, 98, 111, 143
  autosomal, 2
Linked loci, 92
Locus, 16
Lwoff, 3
Lyon hypothesis, 3, 4, 14, 78
  evidence for, 15
Lyonization, 15

Malaria, 2, 75, 109
Malignant hyperpyrexia, 109
Map distance, 93
Map unit, 93
Mapping, chromosomal, *see* Chromosomes
Marfan's syndrome, 90, 137
Marker genes, 13, 93
Mating, *see* Assortative mating
McClintock, 3, 66
Mediastinum testis, 23, *24*
Medulla of ovary, 27
Meiosis, *18*, 20, 31, 50 *see also* Division, cell
  meiosis I, 21, 28, 31
  meiosis II, 22, 28, 31
Membrana granulosa, 28, *29*, 29
Mendel, Gregor, 1, 4, 142, 149
  laws of inheritance, 1
    first, of segregation of alleles, 1, 82
    second, of independent assortment, 1, 91, 143
Mendelian genetics, 142
Mendelian inheritance, 81, 82
Metabolic block, 100
Metacentric, 8
Metaphase, *7, 8, 9*, 9, 20
  in meiosis, *21*, 22
  in mitosis, *18, 19*, 20
Migration, 128
Milstein, 3
Miscegenation, 128
Mitosis, *18*, 19, *see also* Division, cell
Mixed lymphocyte culture, 117
Mongolism, *see* Down's syndrome
Monoclonal antibodies, 3
Monod, 3
Monosomy *42*, *see also* Chromosomes, abnormal
Morgan, 3, 149
Mosaicism, 9, 15, 45
Mosaics, 38, *45*
Mouse,
  dwarf, 78
  pygmy, 78
Mucopolysaccharidoses, 86, 100, *102*, 140
Muller, 3
Multifactorial inheritance, 96, 98
Multiple alleles, 96, 98, 111
Murine leukaemia virus, 69
Mustard gas, 75, 79

Mutagenic agents, 75, 79
Mutant, 69
Mutation, 50, 51, 69, *74, 75*, 79, 100, 128, 136, 143
  chromosomal, 70
  frameshift, 72, *73*
  germ-cell, 70, 79
  induced by X-ray, 70, 79
  intragenic, 70
  point, 71, 79
  rate, 70
  research, 70
  somatic, 70, 79
  spontaneous, 70, 79, 84

Nail-patella syndrome, 92
Nathans, 3
Natural selection, 32, 128
Neurofibromatosis, 70
Neurospora, 144
Neutrons, 75
Niemann-Pick disease, 100, *102*
Nirenberg, 3
Nitrofurantoin, 109
Nitrous acid, 75
Nondisjunction, *23*, 23, 41, 49, 55, 71
Nonpenetrance, 90, 98
Nonrandom mating, 127
Normal distribution curve, 96, *97*
Notch wing, 143, *144*
Nowell, 3
Nucleolar organiser, 8, 55
Nucleoside, 51, *52*
Nucleotide, 51, *52, 53*
Nucleoprotein, 16, 78
Nucleosome, 16

Ochoa, 3
Oestrogen, 28
One gene one enzyme concept, 2, 100
Oogenesis, 28
Oogonia, 27
Operon, 59, *60*, 67
Organ transplantation, 111
Osteogenesis imperfecta, 84, 90
Otto, 2
Ovarian cycle, 30
Ovarian dysgenesis, *see* Turner's syndrome
Ovary, *27*, 27
Ovum, 5, *27, 29*, 91

Panmixis, 127
Pattern baldness, 95
Pedigree analysis, 142
Pedigree charts, 82
Penetrance, 90, 98
Penicillamine, 140
Peyer's patches, 112
Pharmacogenetics, 100
Phenacetin, 109
Phenobarbitone, 140
Phenotype, 50, 81, 82
Phenylalanine hydroxylase, 91
Phenylketonuria, 3, 72, *85*, 86, 91, 100, *102*, 103, *104, 134*, 136
Phenylthiocarbamide, taste sensitivity to, 93
Philadelphia chromosome, 12, 37
Photoreactivation mechanism, 76
Phytohemagglutinin, 9
Placentocentesis, 139
Pleiotropism, 81
Pleiotropy, 81, 91, 98
Point mutation, *see* Mutation
Polyadenylation,61, 68
Polycystic kidney, 97
Polydactyly, 2, *85*, 90
Polygenic, 96
Polygenic inheritance, *see* Inheritance, polygenic
Polymorphism, 111
Polynucleotide, 52
Polynucleotide ligase, 76
Polypeptide, 59, *60*, 62
Polyploid, 6, 41
Polyposis coli, 140
Porphyria, 84, 86, *102*, 109, 140
Precocious puberty, 94
Primaquine, 108, 109
Primary construction, *see* Centromere
Primary oocyte, 28
Primordial follicle, 28
Proband, 82
Progeria, 76
Prophase,
  in meiosis, 2, 21
  in meiosis I, stages of, *21*, 21
    diakinesis, *21*, 22
    diplotene, *21*, 22
    leptotene, *21*, 22
    pachytene, 10, *21*, 22
    zygotene, *21*, 22
  in mitosis, *18, 19*, 20
Proposita, 82, *83*
Propositus, 82, *83*
Protein structure, 64
Protein synthesis, 50, 58, 59, 79
  genetic control of, 59
Pseudo cholinesterase, abnormal, 108
Puffs, 78
Purine, in nucleic acids, *51*, 51, 71
Pyloric stenosis, 97, 137
Pyrimidines, in nucleic acids, *51*, 51, 71

Quinacrine fluorescence, 5
Quinacrine mustard, 10

Radiography, 138, 141
Random genetic drift, 128
Random mating, *see* Panmixis
Recessive, 70, 82
Recombination, 22, 51, 93, 98, 144
Recombinant clones, 77
Recombinant DNA cloning, 61, 79, 142, *148*, 148
Reduced penetrance, 90
Relaxin, 28
Repression-derepression, mechanism of, 78
Repressor protein, 60
Residual body of Regnaud, *26*, 26
Restriction enzymes, 3, 61, 69, 79, 147
Rete testis, 23
Retinoblastoma, 13
Reverse transcriptase, 68
Rheumatic fever, 117
Rheumatoid arthritis, 117
Rheumatoid factor, 117
Ribonucleic acids, *see* RNA
Ribosomes, *57, 58*, 59
  in protein synthesis, 59
Ridge count, 132
RNA, 3, 50, 51, *53*, 58, 79
  adaptor, 62
  cap, 62
  heterogenous, 57
  hnRNA, 68
  messenger (mRNA), 50, *57, 58*, 58, 59, *60, 63*, 68, 79
  nuclear, 66
  ribosomal, 55, 57, 58, 79
  soluble, 59, 79
  transfer (tRNA), 50, *57*, 57, *58*, 58, 59, 62, 79
RNA directed DNA synthesis, 69
RNA polymerase, 58, 59, 60, 61
Roux sarcoma virus, 69

Sanger, 66
Satellite, 8, 71
Screening, 103, *140*, 141
Secretor, 120
Secretor trait, ABO, 92, 96, 120
Seminiferous tubules, 23, *24, 25*
Sertoli, cells of, 23, *24*, 25
Sex chromatin, 13, 14
Sex chromosomes, *see* Chromosomes
Sex conditioned, 95, 98
Sex controlled, 95, 98
Sex influenced, 95, 98
Sex limited traits, 94, 98
Sex linkage, *see* Linkage

Sexual dimorphism, 94
Shope papilloma virus, 140
Sickle cell anaemia, 69, 87
Sickle cell trait, 75
Signal sequences, 64
Simian crease, 44, *45*, 130
Smith, 3
Southern hybridization, 69
Sperm, 5, 24, *25*, 91
Spermatocytes,
  primary, *24*, 24, *25*, *26*
  secondary, *24*, *25*, 25, *26*
Spermatocytogenesis, *24*, 24, *25*
Spermatogenesis, human, *24*, 24, *25*, *26*
Spermiogenesis, 24, 26
Spermatogonia, *24*, 24, *25*, *26*
Spermatozoa, 23, *24*, *25*, *26*
Spina bifida, 137
Structural gene, *see* Gene
Stratum granulosum, 28
Succinylcholine, 108, 109
Sugar, pentose, *51*, 51, 52
  deoxyribose, 51
Sulfonamides, 109
Swiss-type agammaglobulinaemia, *112*, 117
Synteny, 92

TATA box, 61, 63
Tatum, 2, 3, 100
Tay-Sachs disease, 100, *101*
Telomere, 8
Telophase,
  in meiosis, *21*, 22
  in mitosis, *18*, *19*, 20
Temin, 3
Teratogens, 78, 136
Terminator codon, 59, 63, 67, 71
Terminators, 64
Test cross, *see* Back cross
Testicular feminization syndrome, 88
Testis, 23, *24*
Testosterone, 23
Tetraploid, 41
Thalassemia, 69, 75, 108
Theca externa, 28, *29*
Theca interna, 28, *29*
Thymine, *51*, 51, *53*
Thymidine kinase, 144
Tissue typing, serological, 116
Tjio, 3
Toxaemia, 137
Trait, 50, 81, 82, *see also* Inheritance, Pedigree
  polygenic, 96
  quantitative, 96
  quasicontinuous, 97, 98
  single-gene, 97
  threshold, 97
Transcription of DNA to RNA, *57* 58, 59, 60, 61, *63*
Transition, 71
Translation, 62, 63
Translocation, *see* Chromosomal aberration
Transplantation, 114
Transversion, 71
Trinucleotide, 52
Triploid, 41
Triradius, axial, *130*
Trisomy, *42*, *43*, 43, 45, 49, *132*, 136
Turner's syndrome, 13, 32, 33, 34, 39, 43, 45, *46*, *47*, 137
Twins, 126, 128, *129*, 133
  classification of, 128, 133
  zygosity, determination of, 129

Ultrasonography, 138
Universal donors, 118
Universal recipients, 118
Uracil, *51*, 51

Vitamin-D-resistant rickets, 87, 89
von Tschermak Seysenegg, 1
von Willebrand's disease, 70

Watson, 3
Weinberg, 2
Wilkins, 3
Wilson's diseases, 86, 100, *102*, 140
Wiskott-Aldrich syndrome, 117

X Chromosome, *see* Chromosome, sex
Xenograft, 115
Xeroderma pigmentosum, 75
X-linked traits, 70
X-rays, 75, 79, 143

Y Chromosome, *see* Chromosome, sex

Zona pellucida, 29
Zygote, 6, 91
Zygotene, 21

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